# A CORRELATIVE STUDY ON BONE MARROW ANGIOGENESIS WITH BONE MARROW FIBROSIS AND SPLENOMEGALY

**Dissertation submitted in**

**Partial fulfilment of the regulations required for the award of**

**M.D. DEGREE**

**In**

**PATHOLOGY – BRANCH III**

**THE TAMILNADU**

**DR. M.G.R. MEDICAL UNIVERSITY**

**CHENNAI**

**APRIL,2013.**

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I**, Dr.K.Anbu lenin** solemnly declare that dissertation titled, **"A CORRELATIVE STUDY ON BONE MARROW ANGIOGENESIS WITH BONE MARROW FIBROSIS AND SPLENOMEGALY"** is a bonafide work done by me at Coimbatore Medical College during Aug 2011-July2012 under the guidance and supervision of **Prof.Dr.M.Murthy,M.D.,**Professor&Head,Dept.of Pathology,Coimbator Medical College,Coimbatore.

The dissertation is submitted to **The Tamilnadu Dr.M.G.R.Medical University,**towards partial fulfillment of requirement for the award of **M.D.Degree in Pathology (BRANCH – III ).**

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# CERTIFICATE

This is to certify that the dissertation entitled **"A CORRELATIVE STUDY ON BONE MARROW ANGIOGENESIS WITH BONE MARROW FIBROSIS AND SPLENOMEGALY"** is a bonafide work done by **Dr.K.Anbulenin**, Post Graduate student in Department of pathology,Coimbatore Medical College,under the supervision and guidance of **Dr.M.Murthy, M.D,** Professor & Head, Department of pathology, Coimbatore Medical College, Coimbatore, in partial fulfillment of the requirement of **The Tamilnadu Dr.M.G.R.Medical University** for the award of M.D. Degree in Pathology.

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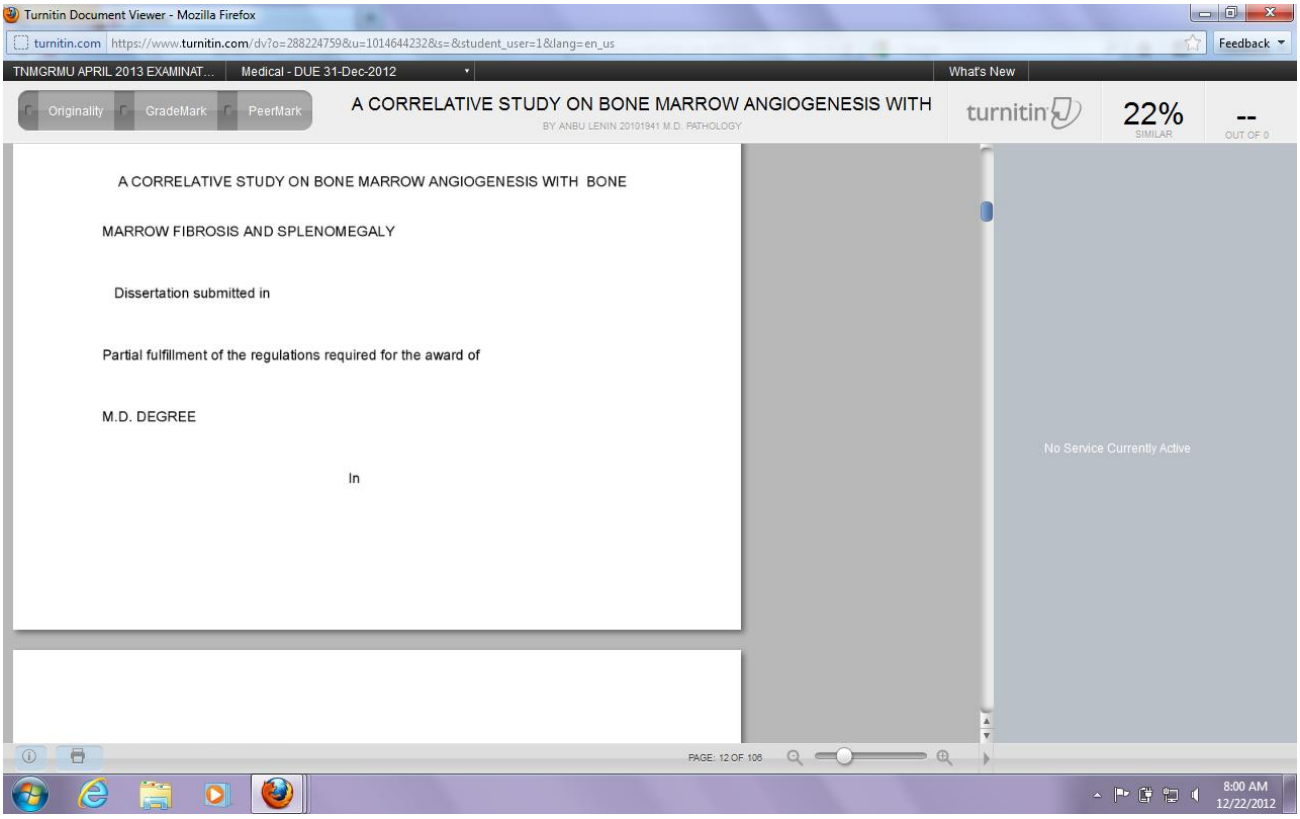


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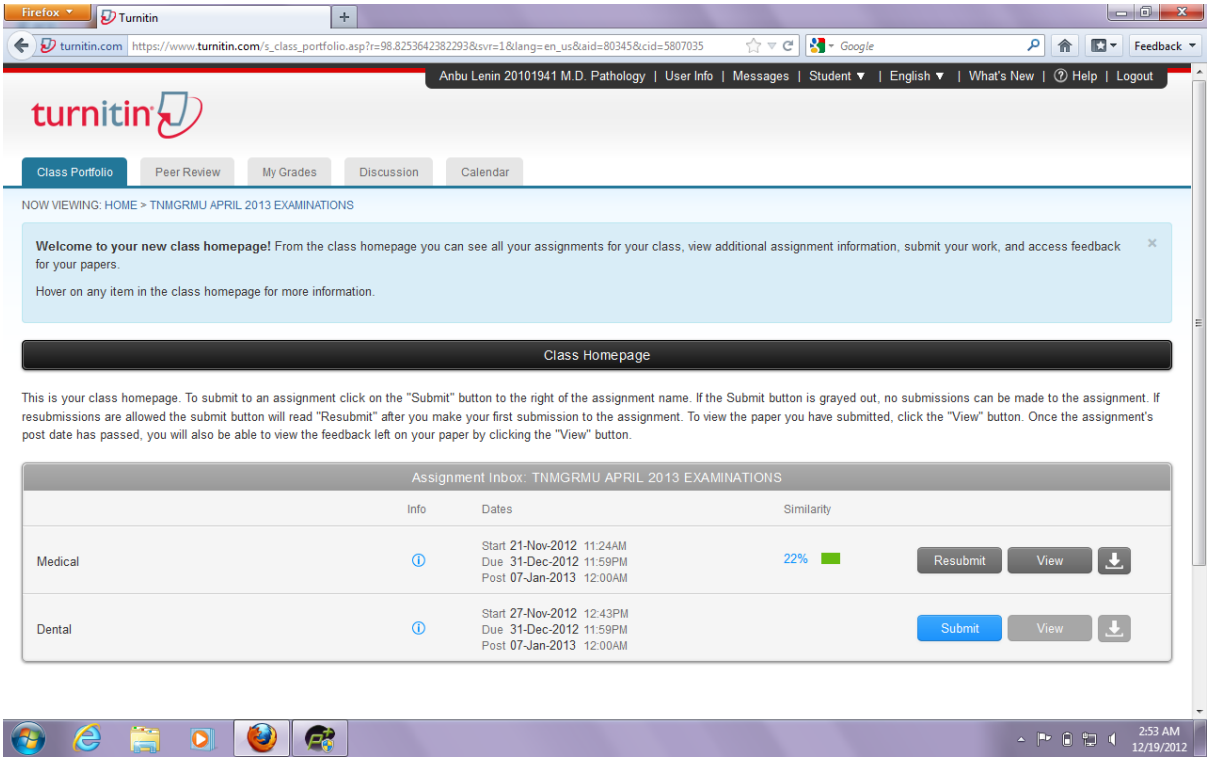
ABBREVIATIONS TGF - Transforming growth factor. PDGF - Platelet derived growth factor. G-6-PD – Glucose 6 phosphate dehydrogenase. IL – Interleukin. IMF – Idiopathic myelofibrosis. MMM – Myelofibrosis with myeloid metaplasia. CML – Chronic myelogenous leukemia. CMML – Chronic myelomonocytic leukemia. CEL – Chronic eosinophilic leukemia. CLL – Chronic lymphocytic leukemia. AML – Acute myeloblastic leukemia. MVD – Mean vessel density. ACKNOWLEDGEMENT I owe a debt of gratitude to Professor.Dr.R.Vimala,M.D., Dean,Coimbatore Medical College,Coimbatore for allowing me to make use of the hospital facilities to carry out this study. I take this opportunity to express my sincere thanks and gratitude...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# ABBREVIATIONS

TGF - Transforming growth factor.

PDGF - Platelet derived growth factor.

G-6-PD – Glucose 6 phosphate dehydrogenase.

IL – Interleukin.

IMF – Idiopathic myelofibrosis.

MMM – Myelofibrosis with myeloid metaplasia.

CML – Chronic myelogenous leukemia.

CMML – Chronic myelomonocytic leukemia.

CEL – Chronic eosinophilic leukemia.

CLL – Chronic lymphocytic leukemia.

AML – Acute myeloblastic leukemia.

MVD – Mean vessel density.

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## AIM OF THE STUDY

To grade bone marrow fibrosis using reticulin stain and to correlate bone marrow microvesseldensity[neoangiogenesis] with reticulin fibrosis and splenomegaly.

## OBJECTIVE

1. To grade Bone Marrow Fibrosis using Gomori's silver stain[Reticulin Stain].
2. To grade Bone Marrow Microvessel density in all the cases of Bone Marrow fibrosis.
3. To analyse the neoangiogenesis in bone marrow fibrosis occurring due to various causes.
4. To correlate Bone Marrow Fibrosis with Microvessel Density.
5. To correlate Bone Marrow Fibrosis and Microvessel Density with Splenomegaly.

# INTRODUCTION

Myelofibrosis is defined as the pathological process characterized by increased deposition of collagen type I and type III in the Bone marrow secondary to release of fibroblast growth factors.

Fibroblast growth factors include PDGF,Epidermal growth factor,Endothelial growth factor,TGF–beta.These factors are present in the alpha–granules of megakaryocytes.

Bone marrow fibrosis usually results from increased stimulation of fibroblasts secondary to release of tumour necrosis factor alpha , IL1alpha and IL–1beta which are produced by bone marrow cells.

Fibroplasia is usually associated with increase in blood flow through the marrow of Intramedullary hematopoiesis,resulting in extramedullary hemopoiesis which frequently affects liver and spleen.

Extramedullary hematopoiesis is associated with poor prognosis.Hence studying the correlation of fibroplasia,spleen size and marrow angiogenesis may be helpful in predicting disease outcome and the patients survival.

# REVIEW OF LITERATURE

## EXAMINATION OF BLOOD ELEMENTS

Careful assessment of the blood elements is often the first and most important step in assessment of hematologic function and diagnosis. Many hematologic diseases are defined by results of blood tests. Examination of well prepared blood smears and hematologic parameters often yields very important diagnostic information,allowing the investigator to have broad differential diagnostic impressions and helping lot in directing additional specific tests.Details on Cellular morphology of various cells present in the blood including erythrocytes, leucocytes (neutrophils, eosinophils, basophils, monocytes, lymphocytes) and thrombocytes and evaluating the variety of parameters relating to cellular size and shape also helps in the diagnosis of specific haematological disorder.

## CELL COUNT AND MORPHOLOGY

Cell counts are most important parameters in evaluating the blood. Cell counts are usually determined either manually or by using a standard automated hematology analyzers. Whether performed by manual means or by using automated methodologies, the accuracy and precision of the count depends on correct dilution of the blood sample, even distribution of blood cells and precise

sample measurement. As blood contains very large numbers of cells, sample dilution must be done for accurate analysis. The type of diluent is dependent on the cell type to be enumerated. Thus, red cell counts require dilution with an isotonic medium, whereas in white cell or platelet counts, a diluent that lyses the more numerous red cells is often used.

Manual counts are done using a light microscope after appropriate dilution of the sample in a hemocytometer, a specially made counting chamber that contains a specific volume. Red cells,leukocytes,and platelets may be counted. Due to the inherent imprecision of manual counting and the amount of technical time required, most cell counting is now performed by using automated analysers.

Evaluation of leukocyte differential count using automated analysers markedly reduces the time and cost of doing routine examinations as well as increasing the accuracy to a CV of 3% to 5% However, automated analyser is highly incapable of accurately identifying and classifying all types of white blood cells and in particular insensitive to atypical or immature cells. Therefore, most of the analyzers will flag the possible abnormal white cell populations, indicating the need for examination by a skilled pathologists for morphological identification[40].Despite giving good results automated analysers sometimes may show spurious increase or decrease in the cell count which may be inherent to disease itself or analyser.This is mainly due to improper calibration of the

instrument.Hence the values given by automated analysers should always be interpreted in context to clinical findings.

Studying the cell morphology mainly red blood cells and white blood cells plays a major role in establishing the correct diagnosis.Red cells exhibit many a number of morphological variation.Red cell morphology evaluation helps in diagnosing many pathological disorder especially primary idiopathic myelofibrosis. Red cells may acquire various shapes including sickle shape in sickle cell disease,acanthocyte in abetalipoproteinemia,burr cells in uremia and tear drop cells in primary myelofibrosis.

**HEMATOPOIESIS AND BONE MARROW**

The initial transient hematopoiesis in human is called as primitive hematopoiesis.This gives rise only to red cell precursors and macrophages, but not giving rise to lymphocytes or granulocytes.This is eventually replaced by definitive hematopoiesis arising from anterior part of Aorta-Gonad-Mesonephros system. This ends after the formation of definitive hematopoietic cells.Soon after the formation of definitive hematopoietic cells they seed the liver,thus making liver an important site of hematopoiesis.Later during the fetal development the hematopoiesis in liver is eventually replaced by bone marrow.

Bones consist of two main parts namely outer cortex and inner medulla.Cortex which forms the outermost portion of the bone is usually

compact and stronger .The inner medulla is usually soft and is composed of cancellous bone.Bone marrow is either red or yellow containing hematopoietic precursor cells or fatty tissue.The marrow cellularity in normal human being is largely dependant on age.In the new born and in childrens generally the entire cavity of the bone marrow is completely replaced by actively multiplying and dividing hematopoietic elements.As the age increases the hematopoietic marrow contracts centripetally,being replaced by adipose tissue resulting in reduced bone marrow cellularity.

**BONE MARROW TOPOGRAPHY**

Marrow is composed of two niches namely osteoblastic niche and vascular niche.Endosteum of mature lamellar bone is lined by osteoblast.Osteoblast play a major role in providing growth factors to hematopoietic stem cell and in differentiation of hematopoietic elements.Vascular niche is constituted by sinusoids.Stromal component of bone marrow provides medium for survival of hematopoietic elements through supplying growth factors.Adhesion molecules provides tight interaction between hematopoietic cells and stromal component.

In the marrow cavity immature cells are usually found in the paratrabecular region.Thus immature cells like blast cells are seen in the paratrabecular region.As the cells mature they migrate towards the sinusoids

afterwhich they enter peripheral circulation.Megakaryocytes are generally seen around the sinusoids.The change in the normal topography of marrow cells is called Abnormal localisation of immature precursors(ALIP) which is characteristically seen in myelodysplastic syndrome and in some myeloproliferative neoplasms.

## MYELOPROLIFERATIVE NEOPLASMS

The myeloproliferative neoplasms are a group of diseases which results from the proliferation of a clone of myeloid cells that are derived from a neoplastic precursor cell.There are many existing evidences saying that even when differentiation of cell is predominantly towards the single lineage ,the disorder should have arisen from multipotent myeloid stem cell or atleast in some cases ,from a pluripotent stem cell capable of giving rise to cells of both myeloid and lymphoid lineages.In chronic myeloproliferative neoplasms ,maturation of the cells is relatively normal and thus cells retains some responsiveness to normal physiological controls.

The myeloproliferative neoplasms differ from myelodysplastic syndrome in that,during early phase of the disease the hematopoiesis is effective with overproduction of cells of atleast one lineage.Dysplastic features either may be totally absent or not very prominent.However ,with the progression of the

disease,hemopoiesis may become more ineffective and dysplastic features may appear.

## BONE MARROW FIBROSIS

The fibroblastic proliferation in marrow is not an intrinsic part of the abnormal clonal expansion of hematopoiesis[(1)].In case of idiopathic myelofibrosis in which G-6PD isoenzyme studies (or) chromosome karyotyping establish monoclonal growth of hematopoietic cells, marrow fibroblasts contain both G-6-PD isoenzymes and do not share the chromosome abnormality[(2)]. This suggests that the fibroblasts differentiate from a primordial cell different from the hematopoietic stem cell and their proliferation and enhanced collagen synthesis is a secondary result of abnormal hematopoiesis.

Fibrosis of bone marrow is not unique to primary myelofibrosis but is associated with many other malignant disorders including Acute myelofibrosis, Acute myeloblastic leukemia, Acute lymphoblastic leukemia, Hairy cell leukemia, Hodgkin's lymphoma, Non Hodgkins lymphoma, Metastatic deposits from breast,lung prostate, stomach and Multiple myeloma. It can also occur in non-malignant conditions including infectious conditions including Tuberculosis and Histoplasmosis, Renal osteodystrophy ,SLE, Scleroderma, Radiation exposure,Thorotrast exposure,etc.

**Various Causes for Bone Marrow Fibrosis**

| Hematologic Disesases | | |
|---|---|---|
| **Myeloid neoplasms** | **Lymphoid malignancies** | **Nonhematological Disorders** |
| 1.Primary idiopathic myelofibrosis | Hodgkin lymphoma | Metastatic carcinomas and Sarcomas. |
| 2.Chronic myelogenous leukemia | Hairy cell leukemia | Autoimmune diseases. |
| 3.CNL | | |
| 4.Myelodysplastic syndrome(MDS) | Non-Hodgkin lymphoma | |
| 5. Acute megakaryocytic leukemia | | Tuberculosis. |
| 6.CMML | Multiple myeloma | Kala-Azar (leishmania)sis) |
| 7.CEL | | Systemic lupus erythematosis. |
| 8. Mastocytosis | Acute lymphoblastic leukemias | AIDS<br>Renal osteodystrophy |

Familial infanti
myelofibrosis

**PRIMARY IDIOPATHIC MYELOFIBROSIS**

Primary marrow fibrosis is a clonal myeloproliferative neoplasm of pluripotent stem cell which is characterised by the proliferation of multiple cell lineage along with progressive increase in marrow fibrosis.It is characterised by splenomegaly,marrow fibrosis,leucoerythroblastic picture and extramedullary hematopoiesis.

In the early phase of idiopathic myelofibrosis ,there may be no increase in bone marrow reticulin fibrosis.When there is increase in bone marrow reticulin

fibrosis ,it is usually accompanied by increase in bone marrow megakaryocytes which will be often morphologically abnormal.

Bone marrow aspiration study often yields a cell poor tap or a hemodilute material.Hence trephine biopsy is always needed to make a definite diagnosis.Smears from successful aspirates may show no abnormality,but usually there is neutrophilic and megakaryocytic hyperplasia.Megakaryocytes are often morphologically abnormal.

## MORPHOLOGY IN MYELOFIBROSIS

| **PREFIBROTIC STAGE** | **FIBROTIC STAGE** |
|---|---|
| PERIPHERAL SMEAR | PERIPHERAL SMEAR |
| 1.No leucoerythroblastosis.<br>2.Minimal anisopoikilocytosis.<br>3.Few tear drop cells. | 1. Leucoerythroblastosis.<br>2.Prominent poikilocytosis.<br>3.Many tear drop cells. |
| **BONE MARROW** | **BONE MARROW** |
| 1.Hypercellular<br>2.Trilineage hyperplasia.<br>3.Megakaryocytic atypia.<br>4.Minimal reticulin fibrosis. | 1.Decreased cellularity.<br>2. Dilated marrow sinusoids.<br>3.Marked atypia in megakaryocytes.<br>4.Reticulin/collagen fibrosis. |

## BONE MARROW FIBROSIS IN MYELOPROLIFEATIVE DISORDERS

Groopman[(3)] first hypothesised that growth factors released from haematopoietic cells in MMM were capable of stimulating marrow fibroblastic

proliferation and suggested that megakaryocyte was the primary source of such proliferation factors.The role megakaryocytes in the development of fibrosis in MMM is further supported by megakaryocytic hyperplasia with dysplasic or necrotic megakaryocytes that characterises this disorder.The supportive evidence to this fact also came from the demonstration of increased fibrosis of Bone marrow in Gray platelet syndrome an inherited disorder of platelet alpha-granule [4-6] and increased fibrosis from the cases of Acute Megakaryocytic leukemia.

Castro-Malaspina and co-workers[7] subsequently showed that megakaryocyte enriched marrow cell homogenates and platelet homogenates induced DNA synthesis by human marrow fibroblast.This group also hypothesized that ineffective megakaryocytopoiesis in MMM results in liberation of excessive amount of growth factors,leading to marrow fibroblast expansion and collagen synthesis[7].

Platelet derived growth factors(PDGF), TGF-beta and epidermal growth factors(EGF), each of which is contained within platelet and megakaryocyte alpha-granules,stimulate marrow fibroblastic proliferation[8-10].

TGF–beta enhances type I and type III procollagen,fibronectin synthesis by marrow fibroblast[9].Kimura and associates[11] showed that myeloproliferative disease fibroblasts are more sensitive to human serum mitogens than normal marrow fibroblasts.

Martyre and collegues[12,13] further examined the possibility that platelet alpha-granule constituents may account for marrow fibrosis in MMM.

The PDGF content of platelets from MMM patients is decreased, indicating that there is leakage (or) release of such growth factors by marrow megakaryocyte[12,13].

TGF-beta enhances fibronectin and collagen type I,III and IV as well as chondroitin or dermatan sulphate and proteoglycan gene expression[14,15]. TGF-beta reduces the production of various collagenase- like enzymes that degrade extracellular ground substances and simultaneously also stimulate the formation of protease inhibitors[16].

Circulating megakaryocyte and platelet express high level of b FGF.These findings may suggest that b FGF also play a crucial role in progressive fibrosis[17,18].

Thrombocytes store and release the calcium binding protein calmodulin[18,19].Extracelular calmodulin is a mitogen for variety of cells including fibroblasts[8,10].Urinary calmodulin excretion is significantly increased in patients with MMM compared to normal individuals without fibrosis[19].

**PROGRESSION OF FIBROSIS IN MMM**

In Wolf and Nieman's series, morphological evidence of progression of fibrosis was present in 1 of 21 cases in which sequential biopsies were

obtained.No connection was observed between bone marrow cellularity, fibrosis and splenic size[56].

In contrast, Lohman and Beckman observed progressive fibrosis in 18 of 20 patients who did not have maximal myelofibrosis at the of initial biopsy[57].Thiele and colleagues presented data to indicate an early prefibrotic subtype of MMM with no (or) minimal medullary reticulin and another phase with conspicuous fibrosis and osteosclerotic changes of the marrow.Based on a careful histomorphometric evaluation of the bone marrow they concluded that in a subset of patients there was a progressive fibro-osteosclerotic process during the evolution of the disease.

Although a steady progression to marrow fibrosis has been demonstrated in patients with prefibrotic stage of myelofibrosis[58,59,60] , fibrosis may remain static (or) diminish in advanced stage of myelofibrosis[61].

Among all the patients of myelofibrosis 20 to 25% of patients are believed to present with the prefibrotic phase of MMM. Most of the patients go on to develop marrow fibrosis over a period of three to four years.Morphological abnormalities of megakaryocytes are reported to be conspicuous during prefibrotic stages and permit these patients to be distinguished from myelodysplasia with fibrosis. The megakaryocytes often appears in clusters adjacent to sinuses and trabeculae.

## DIAGNOSTIC CRITERIAS FOR MMM

Many diagnostic criterias have been proposed for the diagnosis of primary idiopathic myelofibrosis.

## WORLD HEALTH ORGANISATION-DIAGNOSTIC CRITERIA

Diagnosis requires all 3 major and 2 minor criteria.

## MAJOR CRITERIA

1.Presence of megakaryocyte proliferation and atypia, usually accompanied by either reticulin and /(or) collagen fibrosis.

(or)

In the absence of significant reticulin fibrosis,the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterised by granulocytic proliferation and often decreased erythropoiesis (ie.prefibrotic cellular phase disease).

2.Not meeting WHO criteria for polycythemia vera, BCR-ABL + chronic myelogenous leukemia, Myelodysplastic syndrome,or other myeloid neoplasm.

3.Demonstration of JAK 2 V617F or other clonal marker (MPLW515K/L).

or

In the absence of a clonal marker,no evidence that the bone marrow fibrosis (or) other changes are secondary to infection,autoimmune disorder or other chronic inflammatory condition,hairy cell leukemia (or) other lymphoid neoplasm,,metastatic malignancy, (or) toxic chronic myelopathies.

**MINOR CRITERIA**

1.Anaemia.

2.Leukoerythroblastic picture.

3.Splenomegaly.

4.Raised serum lactate dehydrogenase level.

**THE COLOGNE CRITERIA FOR DIAGNOSIS AND STAGING OF MYELOFIBROSIS**

A.No preceding or allied other subtype of myeloproliferative disorders or MDS.

B. Splenomegaly .

C. Thrombocytopenia.

D.Anemia .

E.Leukoerythroblastic blood picture.

F.Histopathology : megakaryocytic plus granulocytic myeloproliferation with large, multilobulated nuclei containing megakaryocytes that show abnormal clustering and definitive maturation defects and

1.No reticulin fibrosis

2.Slight reticulin fibrosis

3.Marked increase in reticulin fibers or collagen fibrosis

4. Osteosclerosis .

Diagnosis and classification of IMF are acceptable if the following combinations are present:

Stage 1: A + B + C + F is consistent with a hypercellular (prefibrotic) stage clinically simulating ET.

Stage 2: A + B + C + D + F - early IMF.

Stage 3: A + B + D + F - manifest IMF.

Stage 4: A + B + D + E + F- advanced IMF which is complicated by osteosclerosis.

## MARROW FIBROSIS AND OTHER MYELOPROLIFERATIVE DISORDERS

Marrow fibrosis can occur in patients with other myeloproliferative disorders, especially Polycythemia vera and CML, and less frequently in primary thrombocythemia[62,63]. In CML progressive marrow fibrosis usually heralds the onset of accelerated disease (or) blast crisis[64].

Myelofibrosis in CML occurs in two different patterns,One in which patients present with CML and significant marrow fibrosis, second in which the myelofibrosis develops late in course of CML. Usually myelofibrosis in CML occurs late at a mean of 36 months, after the diagnosis of CML.

Post polycythemic myeloid metaplasia occurs in 5% to 15% of patients with polycythemia vera. This occurs usually 10 years after the initial diagnosis of polycythemia vera. In such cases previous history of erythropoiesis is very important in the diagnosis of myelofibrosis secondary to polycythemia vera.

## ACUTE MYELOFIBROSIS

A clinically distinct entity called Acute Myelofibrosis differs from MMM in many aspects. Patients clinically present with pancytopenia , fever, absence of significant splenomegaly, absence (or) minimal tear drop cells in blood smear, and marrow fibrosis[66]. Bone marrow in such cases are characterised by the appearance of immature myeloid elements, and the blast cells frequently megakaryocytic phenotypic properties.Acute myelofibrosis is a form of acute megakaryoblastic leukemia.Distinction from MMM is vital, because chemotherapy is needed in case of acute myelofibrosis.

## AUTO IMMUNE MYELOFIBROSIS

The appearance of autoimmune myelofibrosis is indistinguishable from MMM. Auto immune myelofibrosis occurs predominantly in female.Pacquette and colleagues reported that 12% of patients who present with myelofibrosis might suffer from an underlying autoimmune disorder such as SLE.

In addition millarkat and co-workers suggested the existence of an entity,primary autoimmune myelofibrosis(AIMF), a distinct entity unrelated to other well defined autoimmune disorder.They described eight diagnostic criterias including marrow fibrosis (reticulin fibrosis 3(or)4 ), lack of clusters of altered megakaryocytes, lack of dysplasia (or) eosinophilia (or)

basophilia,lymphoid infiltration of marrow, lack of osteosclerosis, absence of spleen enlargement/mild splenomegaly,presence of auto antibodies and absence of disorders associated with myelofibrosis.

## MYELOFIBROSIS AND MYELODYSPLASTIC SYNDROME

A variant of myelodysplastic syndrome with myelofibrosis has been described by pagliuca and co-workers. These patients frequently present with cytopenias and have cellular dysplastic abnormalities indistinguishable from those of other patients with myelodysplasia. Their marrows, however are characterised by the presence of marrow fibrosis , and a striking megakaryocytic hyperplasia, with a predominance of small hypolobated forms, in some cases surrounding fibrosis.

## INFECTIONS AND FIBROSIS

The two most important infectious diseases which results in bone marrow fibrosis are disseminated tuberculosis and Histoplasmosis. Caseating or non-caseating granulomas observed on bone marrow biopsy suggest the presence of these infectious diseases, and should be pursued by culture techniques if possible.

## METASTATIC CARCINOMAS AND FIBROSIS

Secondary myelofibrosis frequently occurs in patients with metastatic carcinoma of stomach,prostate,lung and breast. Successful treatment of primary neoplasms has resulted in the reversal of marrow fibrosis. Demonstration of carcinoma cells in the marrow establishes that metastatic carcinoma is the cause of marrow fibrosis. Lytic bone lesion with myelofibrosis suggests the presence of underlying carcinomas.

## HAIRY CELL LEUKEMIA AND MYELOFIBROSIS

Many a times, Hairy cell leukemias can be confused with MMM. Many patients who had originally been diagnosed as having MMM were shown retrospectively to have had hairy cell leukemia.Hairy cell leukemia can present as pancytopenia with splenomegaly and is associated with a dry marrow tap. The presence of hairy mononuclear cells possessing tartrate-resistant acid phosphatase (or) appropriate phenotype in the peripheral blood (or) marrow should facilitate the differentiation of MMM from Hairy cell leukemia.

## ROLE OF EMPERIOPOIESIS IN MMM

Emperiopoiesis is defined as the random entry of hematopoietic cells into the cytoplasm of megakaryocytes.There is increased emperiopoiesis of neutrophils and eosinophils in MMM leading to liberation of myeloperoxidase-positive granules by engulfed neutrophils which resulted in increased fibrosis[20]. Abnormal p-selectin distribution in megakaryocytes accounted for the selective sequestration of granulocytes by megakaryocytes.

## CELL COUNTS AND MORPHOLOGY IN MMM

Approximately 10% of patients present with pancytopenia because of severe impairment of hematopoiesis affecting each cell lineage,coupled with sequestration in a massively enlarged spleen.Pancytopenia is usually associated with intense marrow fibrosis.

The mean platelet count ranges from 175 to 580 $x10^9$/litre at the time of diagnosis.Individual count can range from 150 to $3215x10^9$/litre.Platelet count is elevated in 40% of patients. Moderate thrombocytopenia is found in one third of the patients. Giant platelets and abnormal platelet granulation are characteristic features of the disease.

The total WBC count show mild increase as a result of granulocytosis. The mean total blood white cell count was 10 to 14 x $10^9$ per litre. Myelocytes and promyelocytes are present in small proportions in most patients, and a low

proportion of blast cells(0.5-2%) may be found in the blood film. Hypersegmentation,hypo-segmentation and abnormal granulation of neutrophils may be present.

Mean haemoglobin concentration ranges from 9.0 to 12.0grams/dl. Anisocytosis and poikilocytosis are constant findings. In all cases tear-drop shaped red cells are seen in sufficient number. Nucleated RBC's may constitute around 2% of nucleated cells in the peripheral blood examination. In some patients hemolysis may be prominent,due to production of red cell auto antibodies.

## CD $34^+$ IN PERIPHERAL BLOOD

Borosi and co-workers reported that number of circulating $CD34^+$ cells tend to increase as the disease progresses and there is a greater chance of evolution to leukemia with more circulating $CD34^+$ cells($>300x10^6$ CD $34^+$ cells/litre) in peripheral blood.

## ANGIOGENESIS

Angiogenesis (or) formation of new blood vessels may be integral to solid tumor growth and metastasis[21]. Quantification and analysis of degree of intra tumoral angiogenesis may furnish some important prognostic information in certain solid tumours[22-27].

In Hematological diseases,Bone marrow is the principal site of disease activity and a easily accessible tissue for the investigation of angiogenesis.The human marrow cavity is usually supplied by a less number of blood vessels.The number of these vessels or the marrow neoangiogenesis is increased in various haematological disorders including acute lymphoid leukemia[28],acute myeloid leukemia[29],Myelodysplastic syndrome[30],Chronic myeloid leukemia[31] and plasma cell disorder[32].Furthermore, microvessel density has been correlated with unfavourable prognosis in multiple myeloma.

Angiogenesis is a important process in the development and progression of solid neoplasms and take part in the phenomenon of metastasis.Thus the grading of microvessel density provides useful prognostic information in haematological neoplasms such as acute leukemia, MDS[34,35]and multiple myeloma[36,37].

Among all the chronic myeloproliferative disorders, angiogenesis is most evident in primary myelofibrosis(MMM)[38,39].

In a study conducted by E.Boveri et al, patients with primary myelofibrosis were found to have significantly higher value of MVD than those with polycythemia vera, Essential thrombocytosis and controls.

Angiogenesis in the bonemarrow is an important factor in the development and progression of IMF.Allogenic stem cell transplant normalizes the increased MVD and disease progression in IMF.

Tumor angiogenesis is believed to be co-ordinated by a fine balance between angiogenic activators and inhibitors. Many molecules have been implicated as positive regulators of angiogenesis, such as VEGF and b FGF.A recent study has shown that there is increased VEGF in the circulation of patient with IMF[41].

Increased expression of bFGF also was detected in circulating megakaryocytes from patients with idiopathic myelofibrosis.

VEGF and b-FGF are potent angiogenic growth factors. It has been suggested that VEGF,by binding to its receptors can inhibit apoptosis of endothelial cells and stimulate endothelial cell migration and proliferation[42].bFGF has been shown to induce endothelial cell proliferation,migration and tubulogenesis[43].Recent data from(Grunewald et al)[44] suggested that the recruited bone marrow derived circulating hematopoietic cells can stimulate endothelial cell proliferation and blood vessel sprouting through increased VEGF secretion.

It is believed that angiogenesis in tumours develops by the phenomenon of blood vessel sprouting from already existing vascular structures through division of fully differentiated endothelial cells[45]. However,recent studies have suggested that angiogenesis also could occur through the de novo formation of blood vessels by bone marrow derived angioblasts (or) common precursor called hemangioblast.[46,47] Gunsilins et al[46] demonstrated that human bone

marrow derived endothelial progenitor cells can integrate into the endothelial cells of blood vessels.

Massa et al[47] demonstrated increased endothelial progenitor cells in patient with IMF. These results suggest that the recruitment and insitu differentiation of bone marrow – derived endothelial progenitor cells also may be essential to promote effective neovascularisation.

## MICROVESSEL DENSITY GRADING

### QUANTITATIVE MORPHOMETRIC MEASUREMENT OF MVD – Niet et al

The histologic sections of bone marrow,were stained first immunohistochemically for CD34.After staining the sections were examined using a light microscope. The entire section was scanned at 100x magninification.Microvessels were identified as endothelial cells appearing as a single cell or cells clustered in nests either with or without lumen.Vessels with thick tunica media,vessels near the periosteum, and sinusoids were excluded.

For quantitative analysis of MVD, 2 photographs were taken from the central portion of each bone marrow section by an investigator(H.N.) without knowledge of the source of the specimen. The MVD was measured using a multipurpose morphometric analysis system proposed by Weibel. Each

photograph were coded and shuffled and then examined randomly,with the observer performing the analysis unaware of the source of the specimen. Random sampling was achieved by using a multipurpose test system that was superimposed on each photomicrograph.This system consists of 84 lines/168 points on a field of 145.5 cm. The presence of microvessels at sample point was recorded. The MVD was estimated by counting the points lying over microvessels, The final result was expressed as a volume percent of microvessels present in the bone marrow using a formula as follows:

$$V = Pa/Pc \times 100\%$$

Pa represents the point number on the microvessel;Pc,the test point number; and V , the volume of the microvessels.

**MEASUREMENT OF MICROVESSEL DENSITY – Mesa et al**

**Three separate methods were used to estimate MVD.**

**METHOD I**

1.Visual scanning of stained slides at all the magnification(100X,400X).
2.Semiquantitative grading for CD34 staining.
3.Accuracy of the method is ensured by review of the slides by two authors.
4.To ensure vessel specific staining morphologic analysis was done.

**METHOD II**

1.Scanning of slide at 100X.
2.Three areas with more number of blood vessels(hot spot) selected.
3.Absolute number of vessels was determined at 400X.
4.Average of three separate visual counts devoid of larger vessels and sinusoids was taken as MVD.

**METHOD III**

In the third and final method, bone marrow MVD was estimated by using computerized image analysis. The 3 hot spots used for the visual count were quantified by computer-based image analysis.A PC- compatible computer running the image analysis software was used for analysis of digitally captured images.By using computerized pixel counting, microvessel surface area was calculated and expressed as the percentage of a bone marrow hot spot occupied by CD 34 staining.An optimized microvessel surface area was then determined by removing the area occupied by fat and expressing the result as a percentage of cellular area occupied by CD34 staining.

**EXTRA MEDULLARY HEMATOPOIESIS**

Spleen is an important site of haematopoiesis in utero and retains its ability to reactivate this process after birth.This can occur as compensatory

erythroblastic hyperplasia in anaemias such as haemolytic anaemia,vitamin-B deficiency anaemia and thalassemia or as a more generalised haemopoiesis often seen in myelofibrosis or other malignant disorders in the bone marrow.

Many of the peripheral blood abnormalities associated with IMF may be attributed to the extramedullary hematopoiesis.Extramedullary hematopoiesis had previously been attributed to the reactivation of quiescent hematopoietic stem cells, which are retained at sites of embryonic hematopoiesis,especially in the spleen[48].

This hypothesis has been questioned because of the observation that spleen,a prominent site of fetal hematopoiesis in humans and by the observation that extramedullary hematopoiesis occurs in wide variety of sites that cannot be accounted by this hypothesis[49-51].

In IMF, after the CD34+ cells (stem cells)exit from the marrow due to their abnormal trafficking patterns,they are filtered by spleen.[52] Ultimately there is an unequal distribution of CD34 cells with two fold greater number being present in the spleen.The myeloid metaplasia of the spleen is characterised by disturbance of splenic architecture with increased proliferation of megakaryocytes and their progenitor cells[52] resulting in extramedullary hematopoiesis and splenomegaly.

In general primitive cells which escape from bone marrow are filtered by the spleen and destroyed.If number of such primitive cells exceeds the capacity

of spleen,these cells are believed to be worked into the peripheral blood, leading to clinical picture of eucoerythroblastosis[53-55].

Splenic size can be assessed by clinical examination and through radiological imaging techniques.The weight of normal spleen ranges from 150-250g.Considerable variation occurs in splenic weight between normal individuals and at various ages in the same individual.At puberty it weighs 200-300g but it starts decreasing after the age of 65 years to 100 to 150g (or) less.In the adult its length is 8-13cm,width is 4.5-7.0cm,surface area 45-80$cm^2$ and volume less than 275$cm^3$.

In the adult an enlarged spleen is palpable when the length of the spleen exceeds 14cm.However measurement of spleen size by physical examination of the abdomen is highly unreliable, as minor enlargement of spleen is often notdetected by palpation and even a grossly enlarged spleen may be missed in an obese person.

As increase in spleen size is associated with increase in bone marrow fibrosis and worse prognosis, it is important to obtain reliable information by radiology through ultrasonic imaging,MRI and CT.

## RADIOGRAPHIC EXAMINATION AND MARROW FIBROSIS

On radiographic examination, characteristic features of MMM are diffuse increase in bone marrow density and increased prominence of the bony

trabeculae.This increased bone density may be patchy and can produce a mottled appearance.

Kaplan and colleagues reported that marrow pattern in the proximal femur of MMM patients correlated with the clinical severity of the disease and that MRI of the proximal femur might be useful in staging and evaluating the progression of the disease process.

# MATERIALS AND METHODS

## STUDY DESIGN

Prospective study.

## STUDY PLACE

Coimbatore Medical College Hospital.

## STUDY PERIOD

August 2011- July 2012.

## SAMPLE SIZE

25 Cases.

## INCLUSION CRITERIA

Newly diagnosed Bone marrow fibrosis of various etiology in patients of all age groups and both sexes admitted to Coimbatore medical college hospital.

## EXCLUSION CRITERIA

1. Already diagnosed patients of bone marrow fibrosis on treatment.
2. Patients with splenomegaly of causes other than bone marrow fibrosis including infections,storage disorders and diseases like amyloidosis,idiopathic splenomegaly.

## METHODOLOGY

Among all the bone marrow trephine biopsy specimens received in the department of pathology,Coimbatore medical college hospital during the study period, 25cases which showed bone marrow fibrosis in routine hematoxylin and eosin stain were selected based upon inclusion and exclusion criteria.

A proforma was used to register various informations about the patients including name,age,sex and clinical profiles including presenting symptoms,past history,treatment history as described in annexure -1.Results of all the available investigations were obtained from various medical records and case sheet.

Hematological Profiles including hemoglobin,total leucocyte count and platelet count were studied using automatic analyser sysmex KX-21. The results obtained were recorded and were used to correlate with the findings of bone marrow trephine biopsy.

Morphological abnormalities of red blood cells,white blood cells and platelets were studied using peripheral smears stained with leishman stain.

Some cases which were accompanied by bone marrow aspiration slides were also studied after staining with leishman stain.Bone marrow aspirates were used to study the following

1.Cellularity.

2.Myeloid:Erythroid ratio.

3.Myelopoiesis.

4.Erythropoiesis.

5.Megakaryocytes.

6.Presence of abnormal cells including dysplastic erythroblasts, micromegakaryocytes, Agranular/hypogranular myeloid precursors, lymphoma cells and cells of metastatic carcinomatous deposit.

7.Parasites.

## BONE MARROW STAINING

Bone marrow sections were cut at a thickness of 4microns from paraffin embedded blocks after fixation and decalcification of bone marrow trephine biopsy specimens.Routine hematoxylin and eosin staining was done.

**Fixative**: 10% neutral buffered formalin.

**Decalcifying agent**: 5-10%nitric acid(6%in our institution).

## PROCEDURE

1. The slides were kept in Xylene for 15minutes.
2. Slides washed in graded alchohol.
3. Slides were washed in water for 5minutes.
4. Slides stained in hematoxylin for 5minutes.

5. Slides were washed in water for 5minutes.
6. Differentiated in 1%acid alchohol(2 dips).
7. Washed in water for 2minutes.
8. Dipped in Lithium carbonate (twice) for blueing.
9. Washed in water for 10minutes.
10. Dipped in 80% alchohol.
11. Stained with eosin for 5minutes.
12. Dehydrated in graded alchohol (80%,90% and then in absolute alchohol).
13. Cleared in xylene and mounted in D.P.X.

**BONE MARROW TREPHINE BIOPSY EXAMINATION**

A Systematic examination of bone marrow trephine biopsy specimens was performed and the following details were obtained.

1. Architecture.
2. Cellularity.
3. Presence of dysplastic megakaryocytes.
4. Megakaryocytic clumping(clustering).
5. Fibrosis.
6. Osteosclerosis.

7.Caseation necrosis.

8.Granulomas.

9.Fungal colonies.

10.Lymphomatous infiltration.

11.Metastaic deposit.

**RETICULIN STAIN**

Reticulin stainining was done after cutting the sections at 4microns thickness from paraffin embedded blocks.

**METAL IMPREGNATION TECHNIQUE AS A CHOICE**

Techniques for the demonstration of reticular fibres may be divided into those using dyes as means of staining and metal impregnation methods.Dye techniques for reticulin demonstration cannot be considered completely reliable,the density of stain being insufficient to resolve the fine fibres.Staining techniques cannot readily differentiate between collagen and reticulin fibres.

**PROCEDURE**

1. Sections were Deparaffinized and brought to water.

2. Sections treated with 1%potassium permanganate solution for 1minute.
3. Rinsed in tap water.
4. Bleached in 2%potassium metabisulfate solution for 1minute,then rinse in tap water.
5. Sensitized with ferric ammonium sulfate for 1minute and washed in distilled water.
6. Impregnated in silver solution for 1minute.
7. Washed in distilled water several times.
8. Reduced in 20%formalin solution for 3minutes and then rinsed in tap water.
9. Toned in 0.2%gold chloride for 10minutes.Rinsed in tap water.
10. Treated with 2% potassium metabisulfite solution for 1minute.Rinsed in tap water.
11. Fixed in 2% sodium thiosulfate solution for 1minute.Rinsed in tap water.
12. Dried in air.
13. Cleared in Xylene.
14. Mounted in D.P.X.

## INTERPRETATION AND GRADING OF RETICULIN FIBROSIS

The stained slides were examined and graded using modified Bauermeister scale. They were given the score of 0-4.

**Grade0:** No reticulin fibres.

**Grade1:**Occassional fine individual fibres and foci of a fine fibre network.

**Grade2:**Fine fibre network throughout most of the section.No coarse fibres seen.

**Grade3:**Diffuse fibre network with scattered thick coarse fibres but no mature collagen.

**Grade4:**Diffuse often coarse fibre network with areas of collagenisation(positive trichrome staining).

## TUMOUR ANGIOGENESIS AND IMMUNOCHEMISTRY

### SECTION CUTTING

The sections were cut at 4microns thickness from paraffin embedded blocks.Section cutting was followed by dewaxing (Incubating sections at 58*c overnight).

### ANTIGEN RETRIEVAL

Many methods have been used for antigen retrieval including pressure cooker method,microwave retrieval,water bath,autoclave and proteolytic enzyme digestion.In our institution we used microwave antigen retrieval method.(Heat mediated antigen retrieval technique).

### BUFFER

Citrate buffer(0.01M) at $p_H$ of 6.

### IMMUNOHISTOCHEMISTRY TECHNIQUE

Two step indirect method.

### CD34 AS A CHOICE OF ANTIBODY

Initially during the study period many antibodies including CD31,Factor VIII,along with CD34 were tried.Results obtained using these antibodies were studied and compared among themselves.The results showed that the staining using CD31 and FactorVIII were less intense(weakly positive).Also in case of antibodies other than CD34 ,staining of primitive cells and blast cells were more in number making the interpretation difficult.Hence the antibody (CD34 )which gave strong positivity was used as antibody of choice.

**PROCEDURE – IMMUNOHISTOCHEMISTRY**

1.Sections are cut at 4microns.Incubate at

58*c overnight.

2.Washed in xylene for 30minutes.

3.Washed in absolute alchohol – 2minutes x 2changes.

4.Washed in tap water for 10minutes.

5.Rinsed in distilled water for 5minutes.

6.Kept in microwave : Medium-10minutes;

High-10minutes.

7.Cooled to room temperature- 20minutes.

8. Rinsed in distilled water for 5minutes.

9.Washed in TBS-5minutes x 2changes.

10.Kept in peroxide block for 10minutes.

12.Kept in power block for 10minutes.

13.Drained and covered in with primary antibody

for one hour.

14.Washed in TBS-5minutes x 2changes.

15.Kept in superenhancer for 30minutes.

14.Washed in TBS-5minutes x 2changes.

17.Treated with S.S label and poly HRP for 30minutes.

18.Washed in TBS-5minutes x 2changes.

19.Treated with DAB and substrate buffer for

5-8minutes.

20.Washed in TBS-5minutes x 2changes.

21.Kept in tap water for 5minutes.

22.Kept in hematoxylin for 30seconds-2minutes.

23.Kept in tap water for 5minutes.

24.Dried in air.

25.Cleared in Xylene.Mounted in D.P.X .

**GRADING OF MICROVESSEL DENSITY**

The slides which were stained for CD34 were graded for microvessel density(neoangiogenesis).The grading was done using visual microvessel density grading system which was introduced by Weidner et al in solid tumours.

**PROCEDURE**

Each of the study slide was first scanned at 100x magnification.Three areas with abundant microvessels were selected and defined as hot spots.The

number of microvessels in each of these hot spots was then determined at 400x magnification.The final MVD number [microvessels per high power(400x) field] was assigned by taking the average of the three separate visual counts.During the process of grading MVD,large vessels and vessels in the periosteum or bone and open sinusoids were excluded.Areas of staining with no discrete breaks were counted as single vessel and presence of lumen was not required.

The results obtained were graded as follows by comparing with age matched controls.After grading,the results were compared among themselves and correlated with bone marrow fibrosis(Reticulin fibrosis) and splenomegaly.

**MVG 1**:Slightly increased.

**MVG 2**:Easy to find and definitely increased from normal.

**MVG 3**:Abundant vessels.

**MVG 4**:Markedly increased.

**SPLEEN SIZE**

Spleen size of the cases were obtained using ultrasonographic examination and were divided into three categories as mild,moderate and severe.The results were correlated with mean vascular density(MVD) and marrow reticulin fibrosis.

## OBSERVATION AND RESULTS

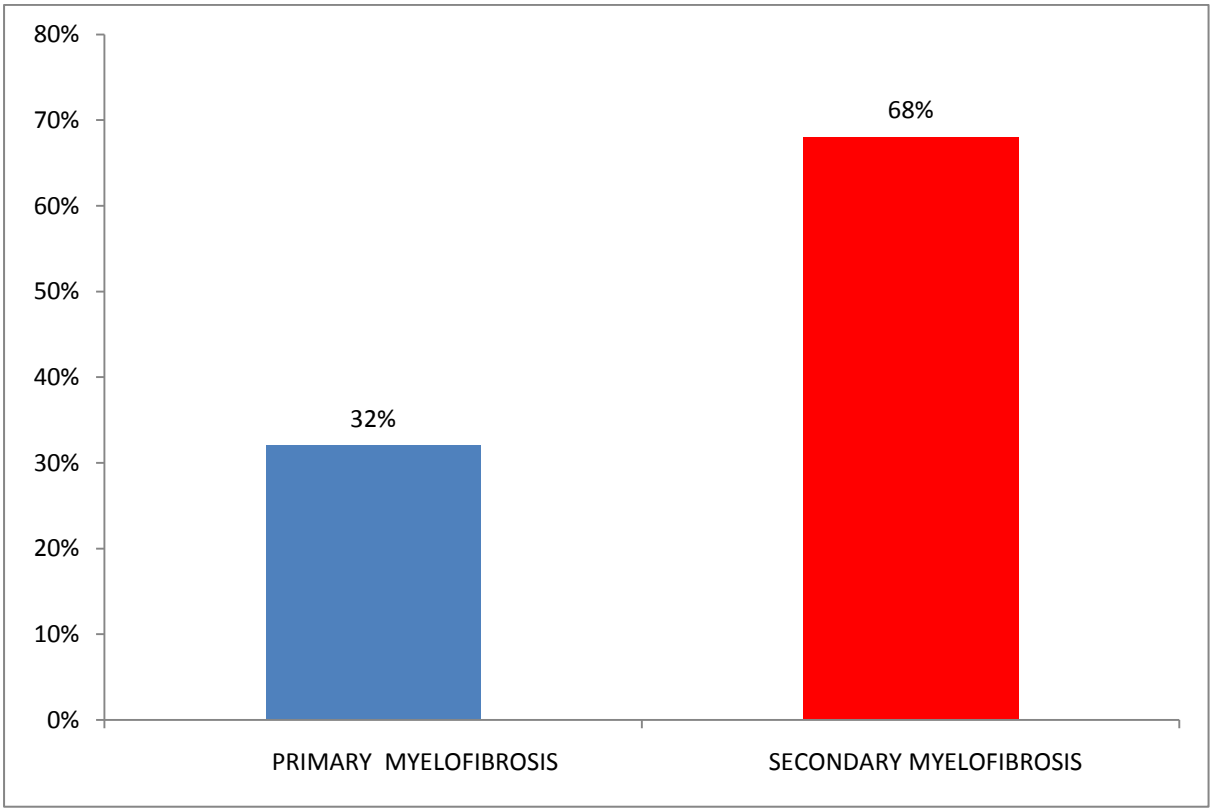
Total of 25cases were studied.Among 25cases,8cases showed features of primary myelofibrosis and remaining 17cases were the cases of secondary myelofibrosis.

**TABLE.1**

| TOTAL NUMBER OF CASES | PRIMARY MYELOFIBROSIS | SECONDARY MYELOFIBROSIS |
|---|---|---|
| **25** | **8** | **17** |
| **PERCENTAGE(%)** | **32%** | **68%** |

Among 25 cases studied totally most of the cases (68%) are the cases of secondary myelofibrosis.Ratio of primary myelofibrosis: secondary myelofibrosis-1:2.This indicates that secondary myelofibrosis is relatively more common than primary myelofibrosis.

## CHART – 1




The above chart comparison clearly indicates that secondary myelofibrosis is more common than primary idiopathic myelofibrosis.

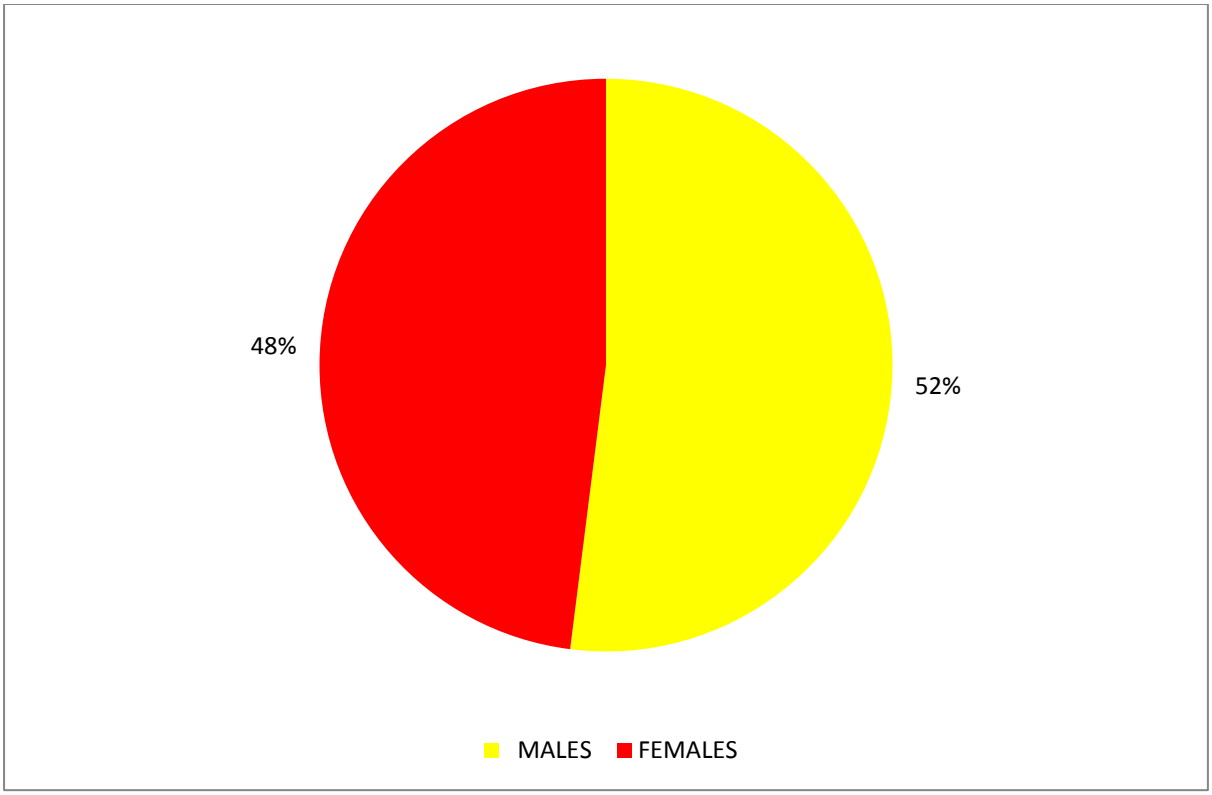
## SEX DISTRIBUTION AMONG ALL THE CASES

### TABLE.2

| TOTAL CASES | MALES | FEMALES |
|---|---|---|
| **25** | **13** | **12** |
| **PERCENTAGE(%)** | **52%** | **48%** |

52% of the total case load was contributed by males.This shows that there is slight degree of male preponderance.

### CHART – 2



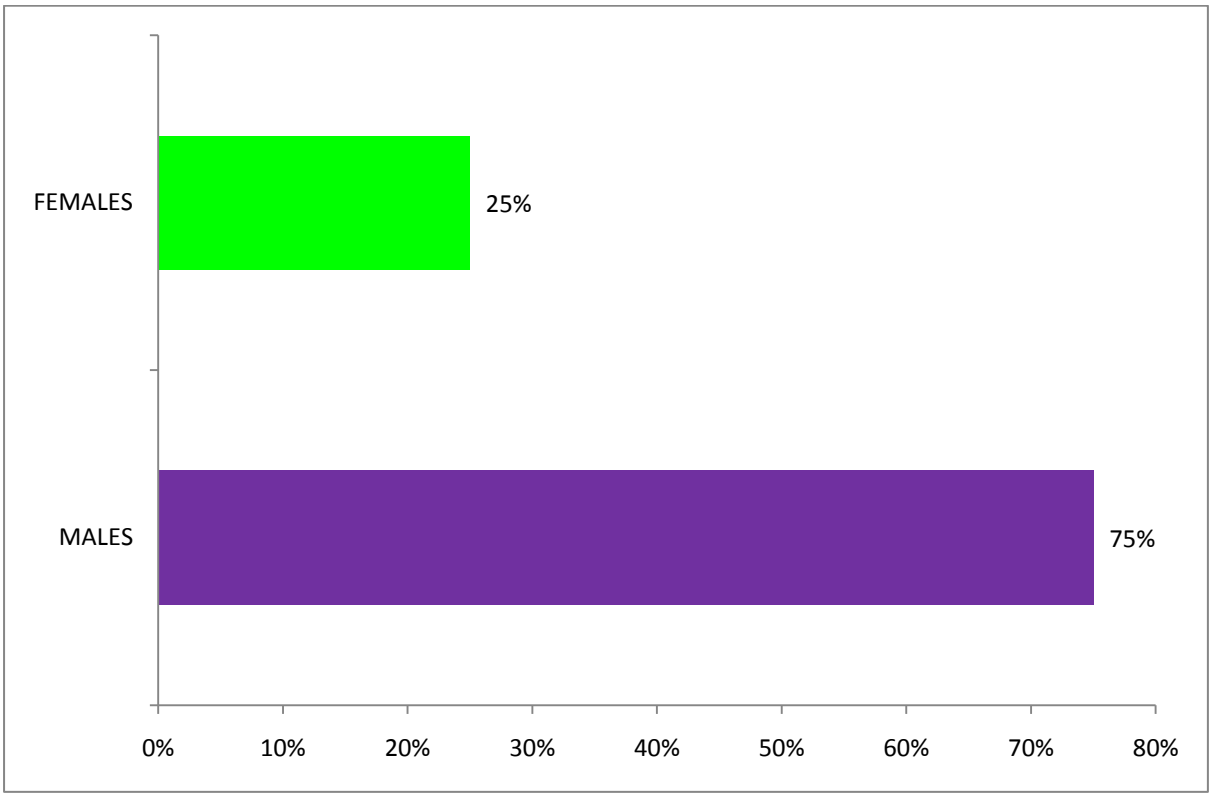
## SEX DISTRIBUTION AMONG PRIMARY MYELOFIBROSIS

### TABLE.3

| TOTAL CASES | MALES | FEMALES |
|---|---|---|
| 8 | 6 | 2 |
| PERCENTAGE(%) | 75% | 25% |

The study included totally eight cases of primary myelofibrosis.In the eight cases studied 75% of the cases were males with male to female ratio of 3:1.

### CHART – 3



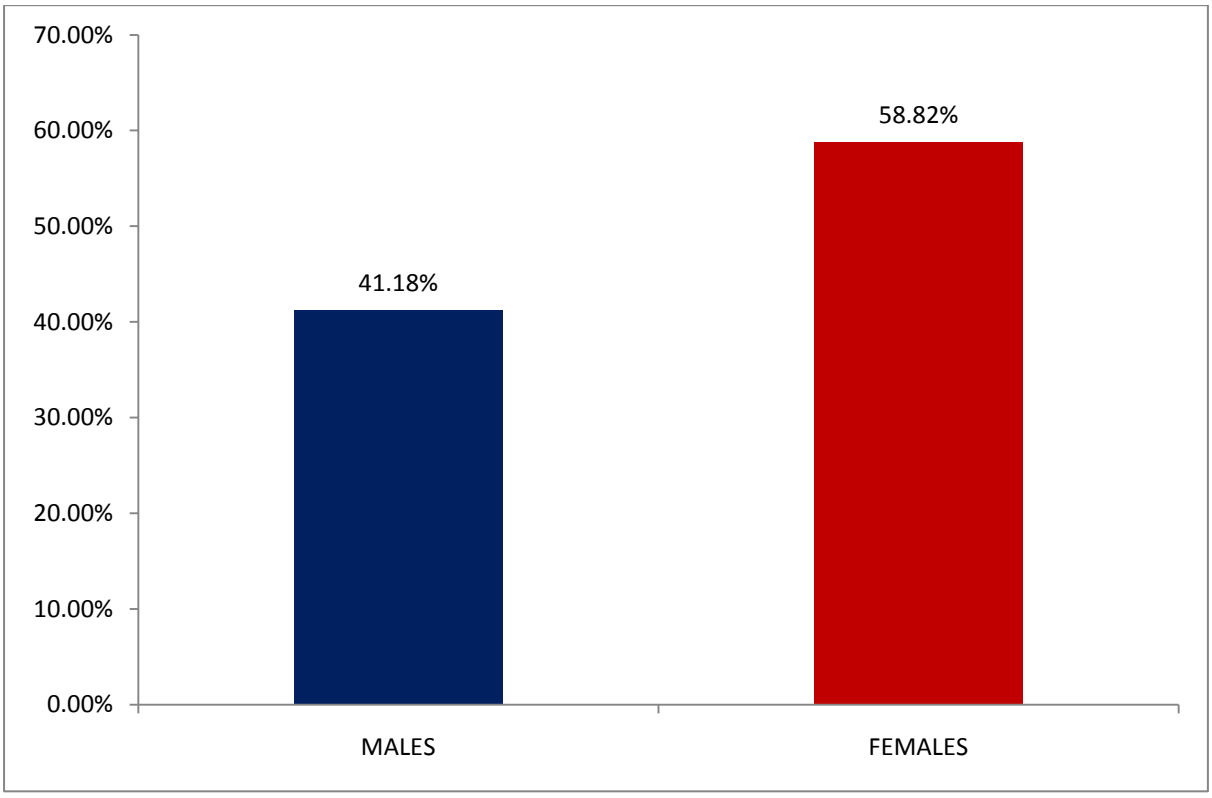
## SEX DISTRIBUTION AMONG SECONDARY MYELOFIBROSIS

### TABLE.4

| TOTAL CASES | MALES | FEMALES |
|---|---|---|
| 17 | 7 | 10 |
| PERCENTAGE(%) | 41.18% | 58.82% |

Maximum number of patients among secondary myelofibrosis were females accounting for 58.82%.This is in contrast to primary myelofibrosis which showed male preponderance.

### CHART – 4

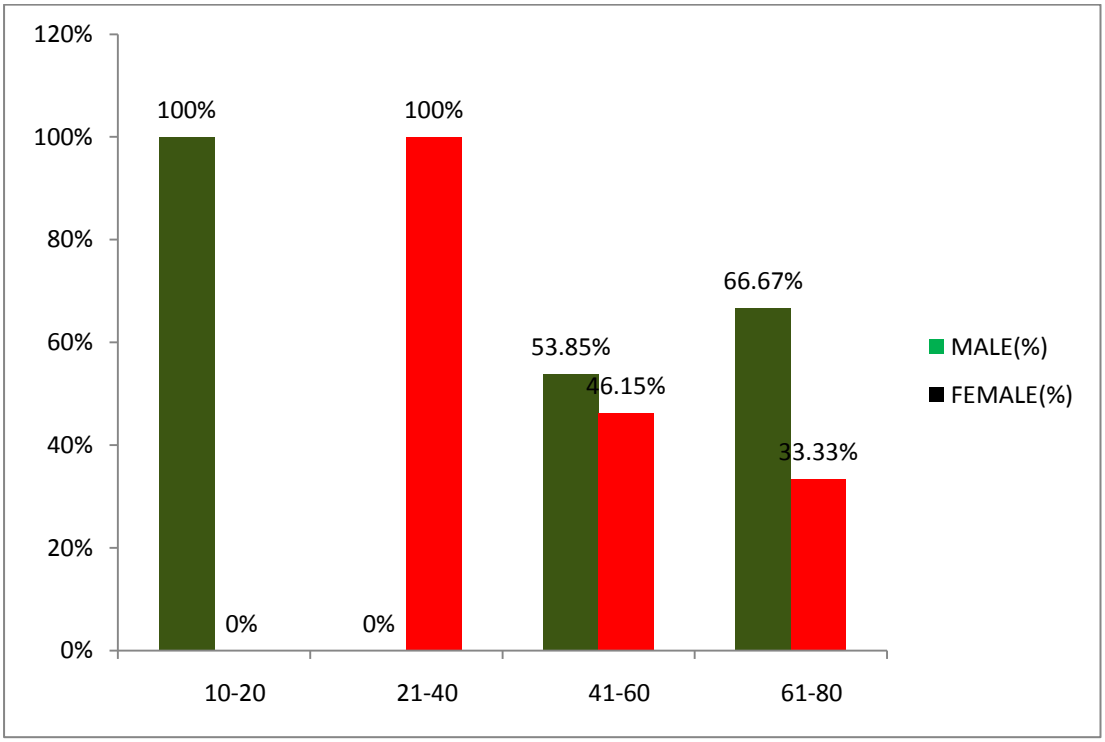


## AGE AND SEX DISTRIBUTION AMONG ALL THE CASES

**TABLE.5**

| AGE (YEARS) | CASES | MALES | FEMALES | TOTAL (%) | MALE (%) | FEMALE (%) |
|---|---|---|---|---|---|---|
| 10-20 | 2 | 2 | O | 8% | 100% | 0% |
| 21-40 | 4 | 0 | 4 | 16% | 0% | 100% |
| 41-60 | 13 | 7 | 6 | 52% | 53.85% | 6.15% |
| 61-80 | 6 | 4 | 2 | 24% | 66.67% | 33.33% |

Age distribution studied among all the cases showed that there were two cases below 20years.Both of the cases were males with one case being case of primary myelofibrosis and other being secondary myelofibrosis.All the cases between 21-40 years were females. The maximum number of cases lie between 41years and 60years.



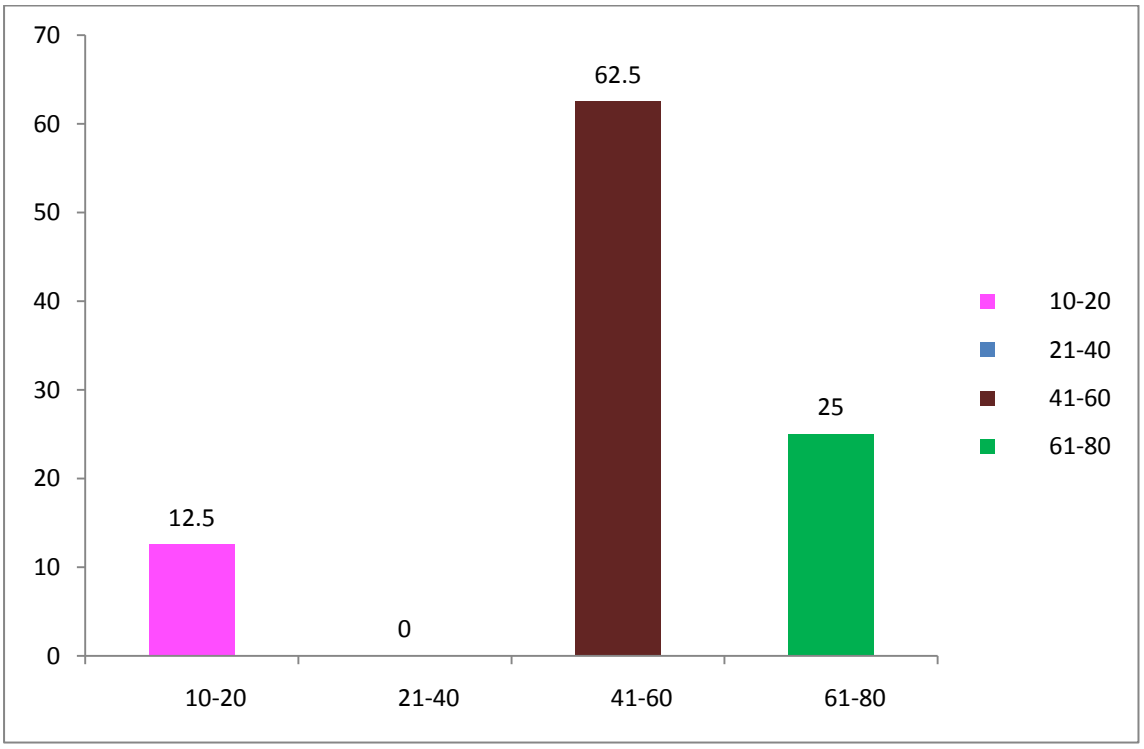
## AGE DISTRIBUTION – PRIMARY MYELOFIBROSIS

### TABLE.6

| AGE(YEARS) | NUMBER OF CASES | PERCENTAGE(%) |
|---|---|---|
| 10-20 | 1 | 12.5 |
| 21-40 | 0 | 0 |
| 41-60 | 5 | 62.5 |
| 61-80 | 2 | 25 |

There were no cases between 21years and 40years.There was one case below 20years.The maximum number of cases were between 41years and 60years.

### CHART – 6



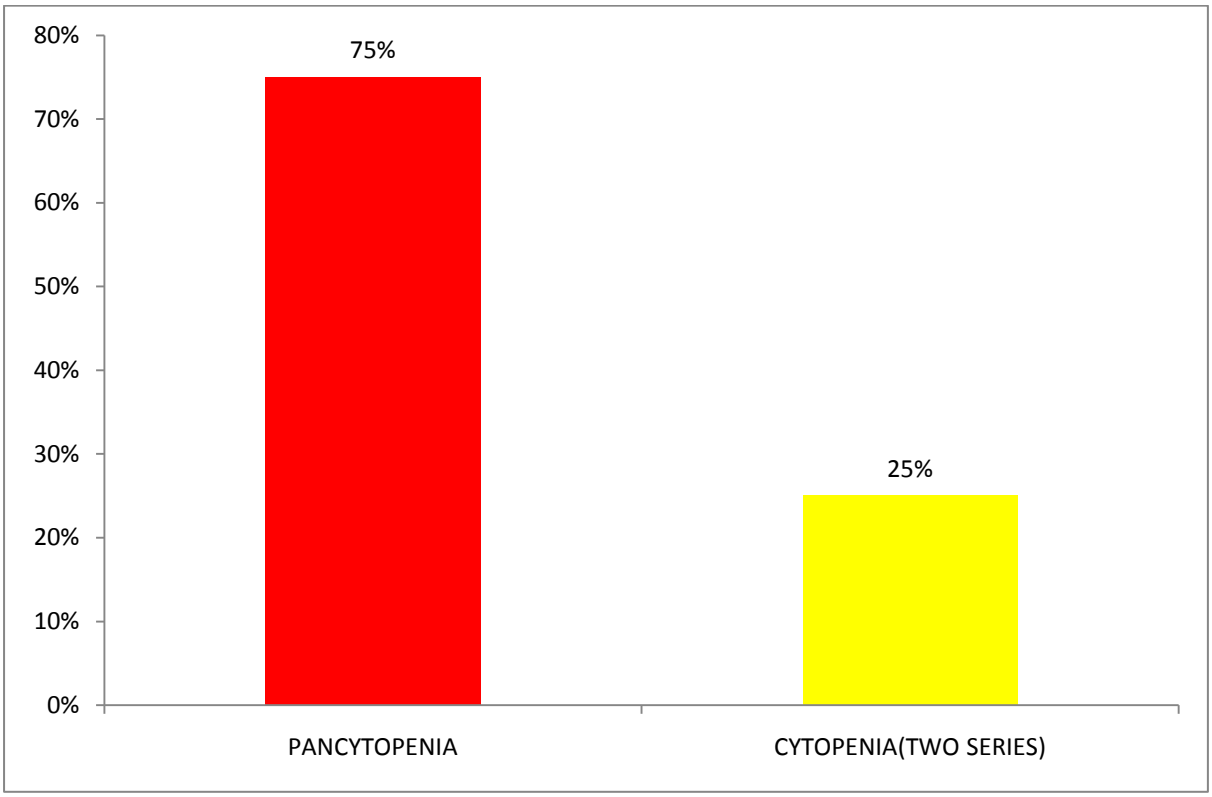
## PANCYTOPENIA AND PRIMARY MYELOFIBROSIS

### TABLE.7

| TOTAL CASES | PANCYTOPENIA | CYTOPENIA(TWO SERIES) |
|---|---|---|
| 8 | 6 | 2 |
| PERCENTAGE(%) | 75% | 25% |

Six cases(75%) among the eight cases studied had pancytopenia.

### CHART – 7



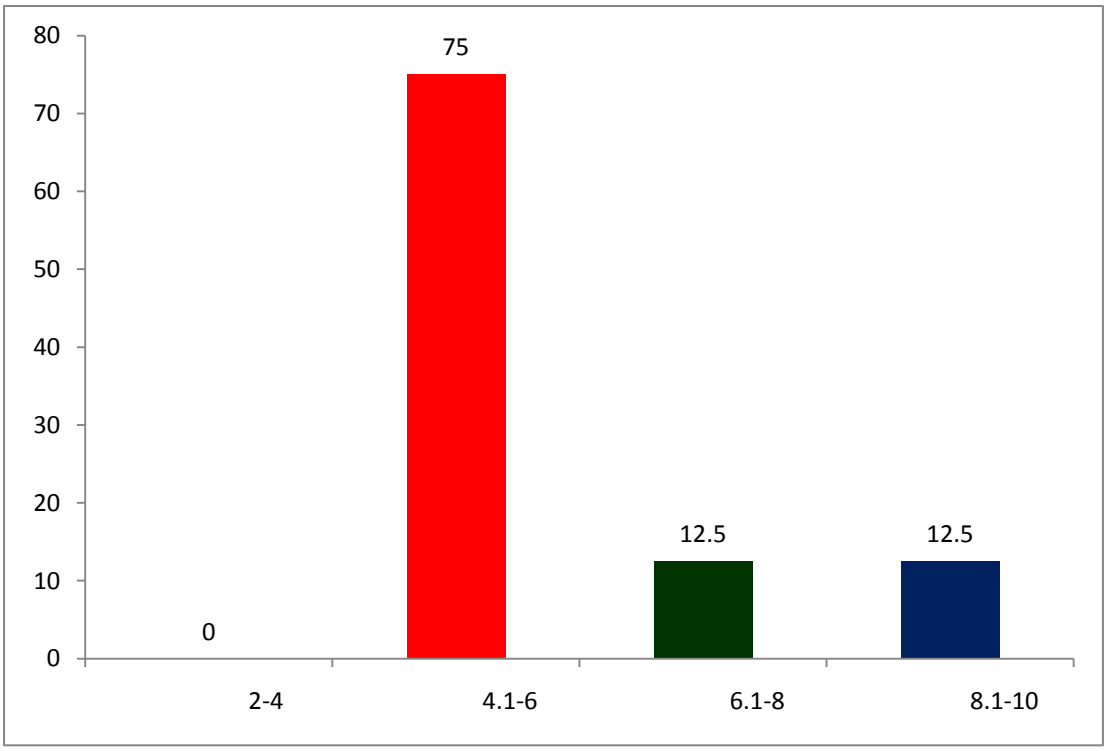
## PRIMARY MYELOFIBROSIS AND HEMOGLOBIN LEVEL

### TABLE.8

| HEMOGLOBIN(grams/dl) | NUMBER OF CASES | PERCENTAGE |
|---|---|---|
| 2-4 | 0 | 0 |
| 4.1-6 | 6 | 75 |
| 6.1-8 | 1 | 12.5 |
| 8.1-10 | 1 | 12.5 |

All the patients of primary myelofibrosis had anaemia.75% of patients had their hemoglobin between 4.1grams and 6.0grams.No patients had their hemoglobin below 4.0grams.

### CHART – 8



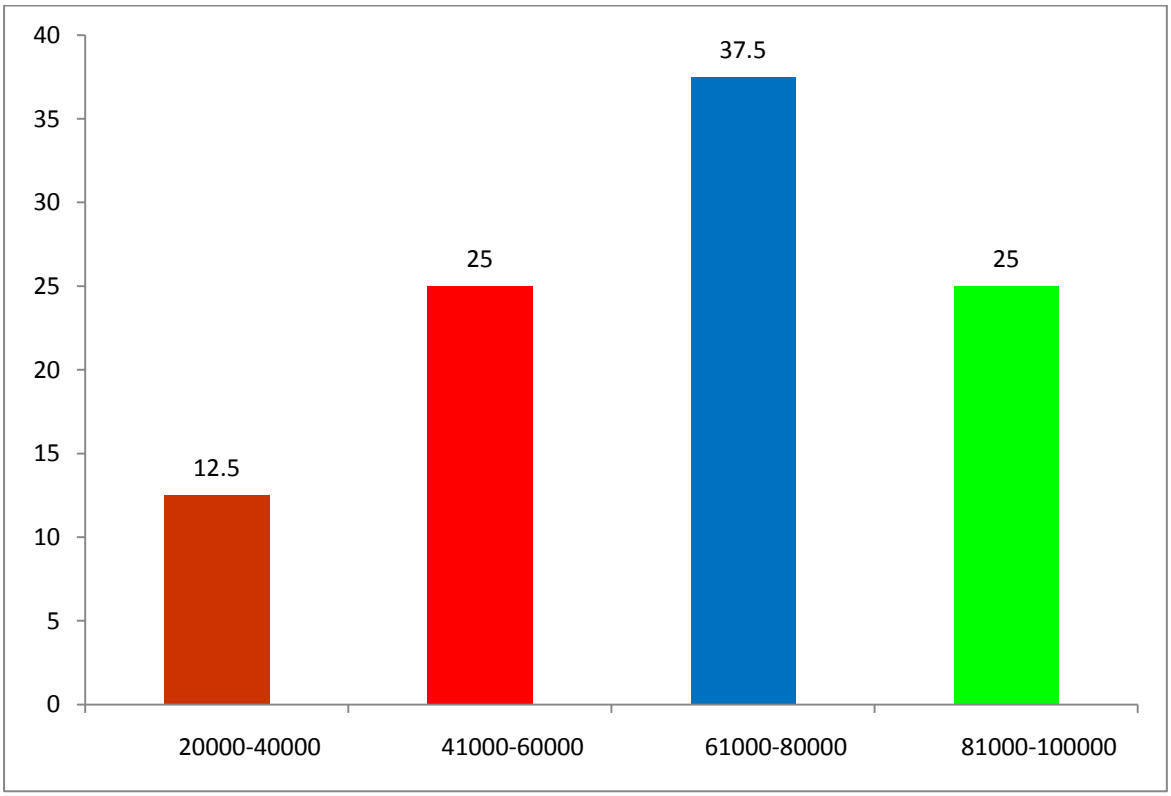
## PRIMARY MYELOFIBROSIS AND PLATELET COUNT

### TABLE.9

| PLATELET COUNT(cells/$mm^3$) | NUMBER OF CASES | PERCENTAGE |
|---|---|---|
| **20000-40000** | **1** | **12.5** |
| **41000-60000** | **2** | **25** |
| **61000-80000** | **3** | **37.5** |
| **81000-100000** | **2** | **25** |

All the cases had thrombocytopenia.Most of the patients (62.5%) had only mild thrombocytopenia.No patients had platelet count below 20,000cells/$mm^3$and hence none of them presented with signs and symptoms of bleeding.

### CHART - 9



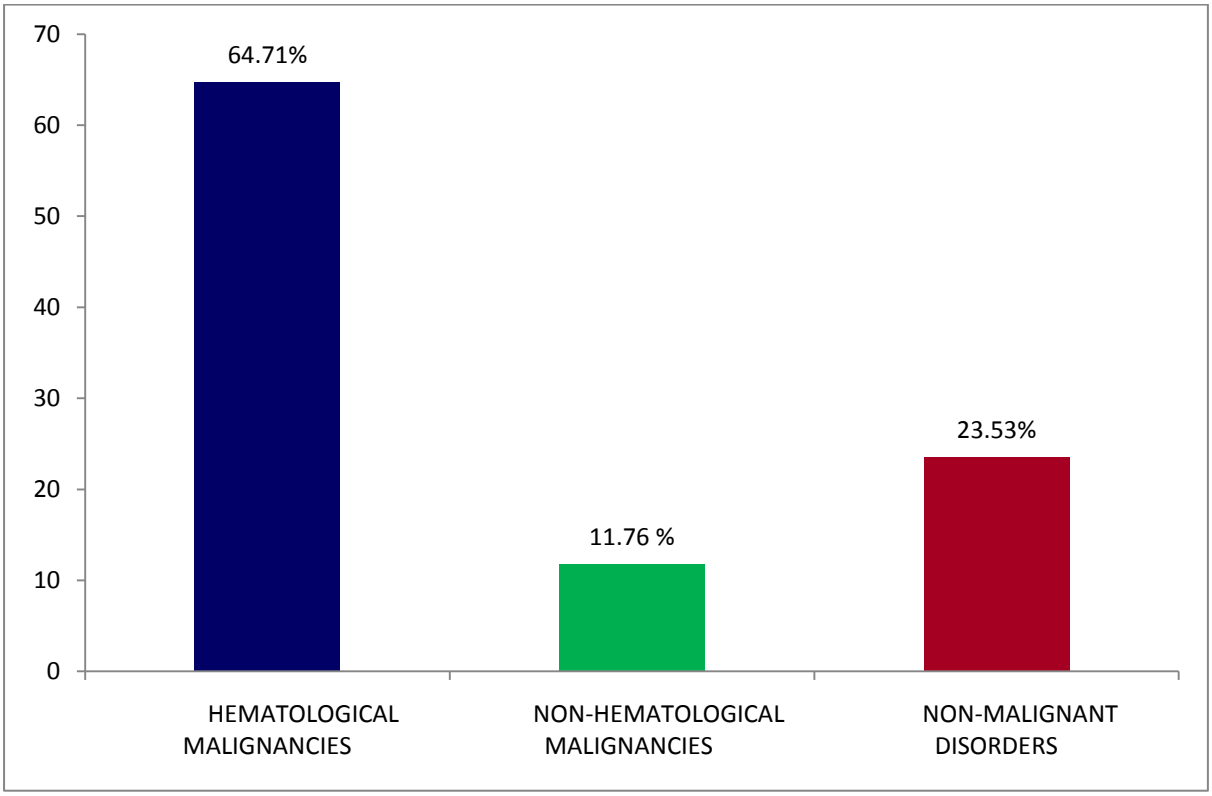
## DISTRIBUTION OF CASES AMONG SECONDARY MYELOFIBROSIS

### TABLE.10

| DISORDERS | CASES | PERCENTAGE |
|---|---|---|
| HEMATOLOGICAL MALIGNANCIES | 11 | 64.71 |
| NON-HEMATOLOGICAL MALIGNANCIES | 2 | 11.76 |
| NON-MALIGNANT DISORDERS | 4 | 23.53 |

Among secondary myelofibrosis most number of cases(64.71%) were due to haematological malignancies including leukemias and lymphomas.Non haematological malignancies mainly metastatic carcinomatous deposit accounts for 11.76%.

### Chart – 10



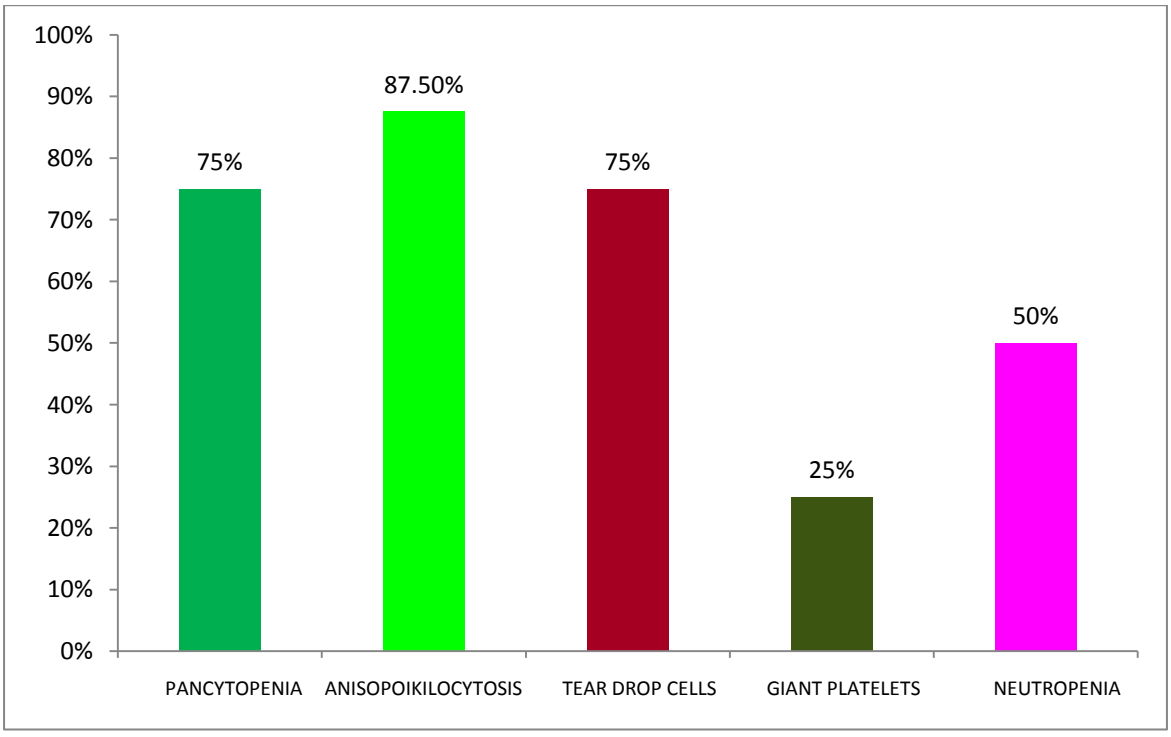
## PERIPHERAL SMEAR AND PRIMARY MYELOFIBROSIS

### TABLE.11

| FINDINGS | NUMBER OF CASES | PERCENTAGE |
|---|---|---|
| PANCYTOPENIA | 6 | 75% |
| ANISOPOIKILOCYTOSIS | 7 | 87.5% |
| TEAR DROP CELLS | 6 | 75% |
| GIANT PLATELETS | 2 | 25% |
| NEUTROPENIA | 4 | 50% |

Peripheral smear examination showed anisopoikilocytosis in most of the cases and it was found to be constant finding in 87.5% of the cases.Tear drop cells which was considered to be pathognomonic feature of idiopathic myelofibrosis was found in six (75%) cases.Neutropenia was seen in 50% of the cases.None of the cases had increase in the granulocyte count.

**Chart - 11**



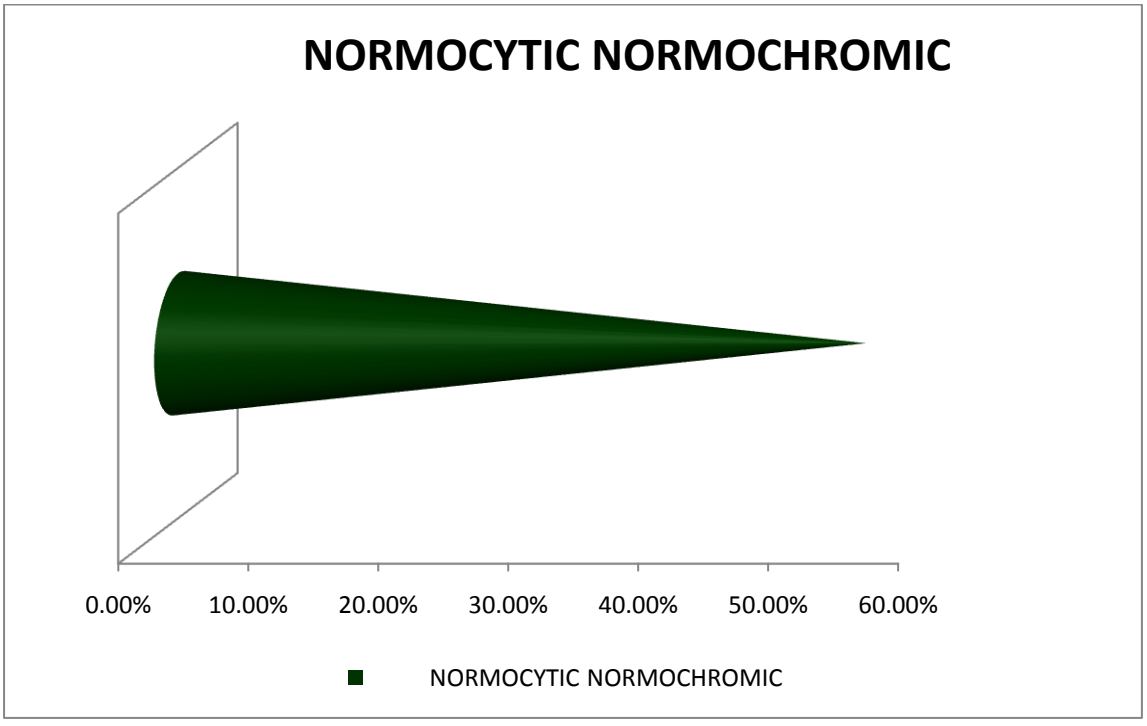
## ANAEMIA DISTRIBUTION AMONG SECONDARY MYELOFIBROSIS

### TABLE.12

| TOTAL CASES | ANAEMIA | MOST COMMON TYPE OF ANEAMIA | PERCENTAGE |
|---|---|---|---|
| 17 | 9 | NORMOCYTIC NORMOCHROMIC | 52.94% |

Among the cases of secondary myelofibrosis 52.94% of cases had anaemia.All the cases had normocytic normochromic anaemia.

### CHART - 12



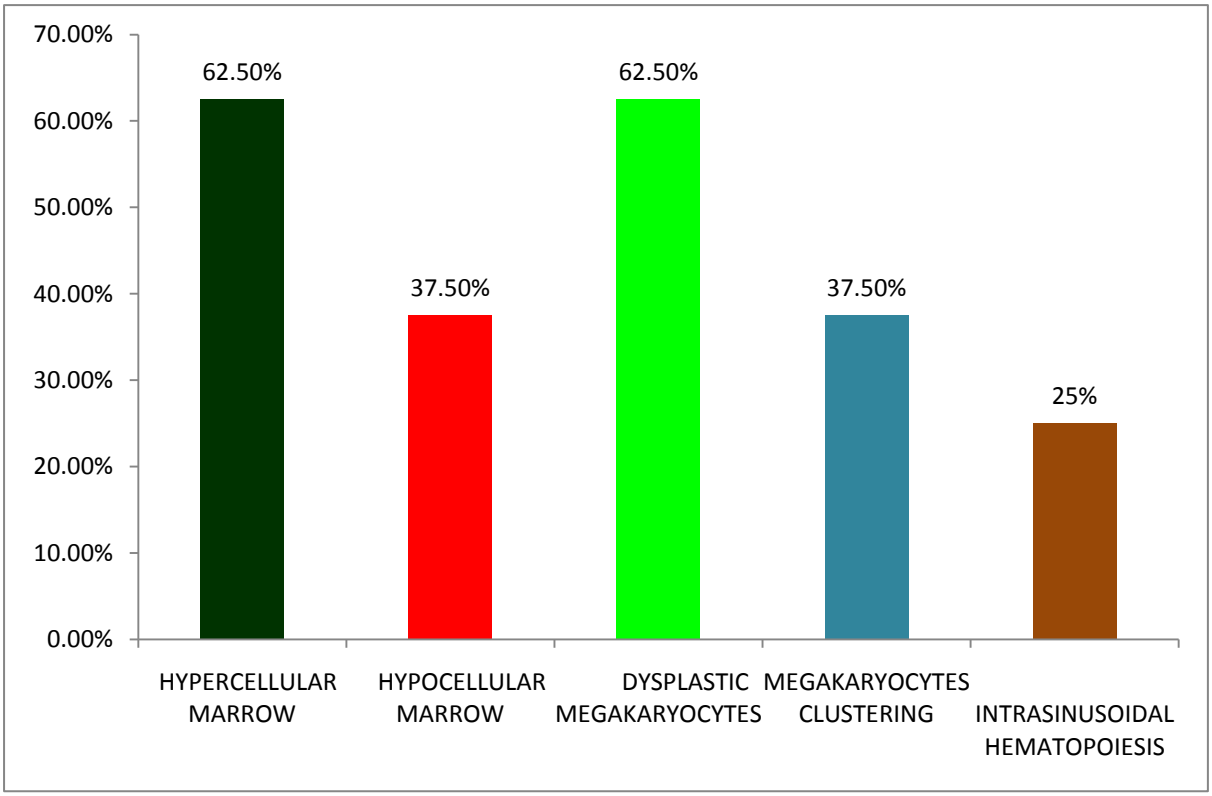
## BONE MARROW MORPHOLOGY AND PRIMARY MYELOFIBROSIS

**TABLE.13**

| BONE MARROW FEATURES | NUMBER OF CASES | PERCENTAGE |
|---|---|---|
| HYPERCELLULAR MARROW | 5 | 62.5% |
| HYPOCELLULAR MARROW | 3 | 37.5% |
| DYSPLASTIC MEGAKARYOCYTES | 5 | 62.5% |
| MEGAKARYOCYTE CLUSTERING | 3 | 37.5% |
| INTRASINUSOIDAL HEMATOPOIESIS | 2 | 25% |

Among eight cases studied 5 patients showed hypercellular marrow and 3 patients showed hypocellular marrow.In the patients of hypercellular marrow 62.5% showed dysplastic megakaryocytes and 37.5% of patients showed megakaryocytic clustering.

**CHART - 13**




Most of the cases showed bone marrow hypercellularity with many of them showing abnormal shaped megakaryocytes.

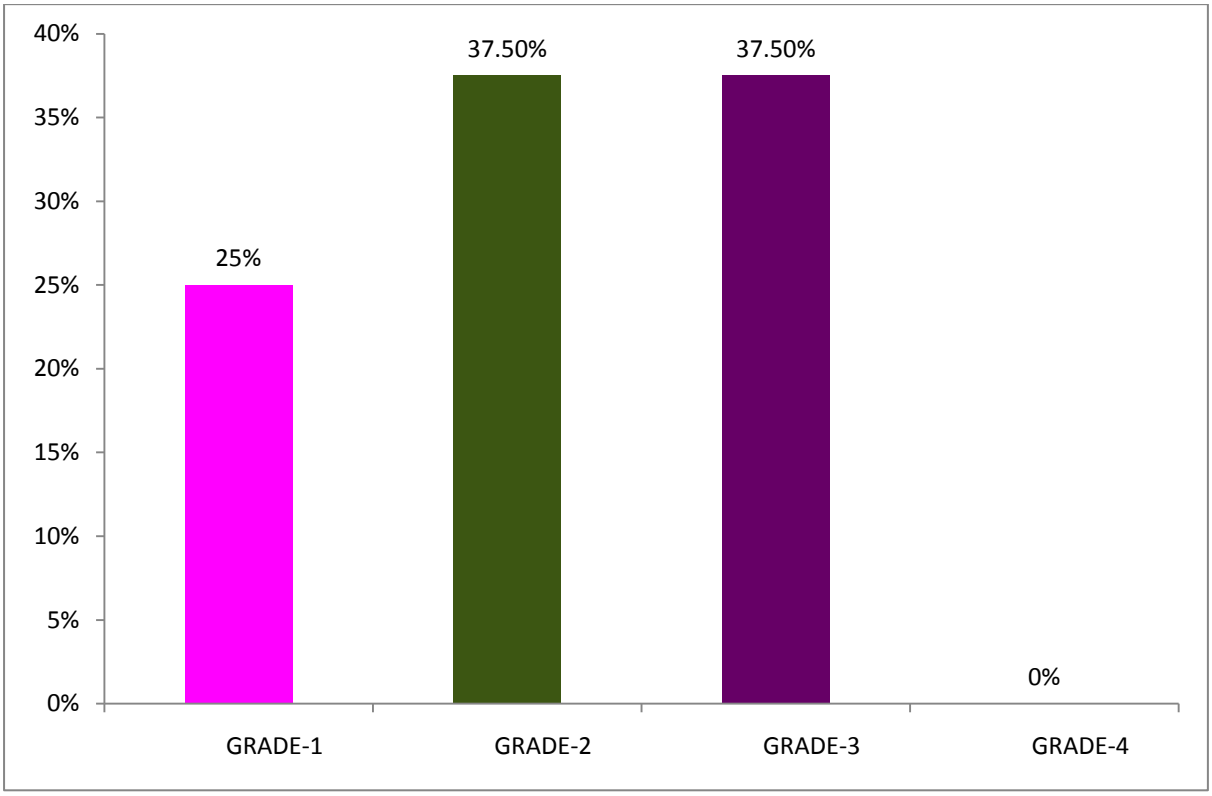
## RETICULIN FIBROSIS AND GRADING IN PRIMARY MYELOFIBROSIS

### TABLE.14

| GRADE OF RETICULIN FIBROSIS | NUMBER OF CASES | PERCENTAGE |
|---|---|---|
| GRADE-1 | 2 | 25% |
| GRADE-2 | 3 | 37.5% |
| GRADE-3 | 3 | 37.5% |
| GRADE-4 | 0 | 0% |

Reticulin fibrosis was evident in all the cases with 37.5% of cases showing grade 2 fibrosis.None of the cases showed collagen fibrosis and osteosclerosis.

### CHART - 14



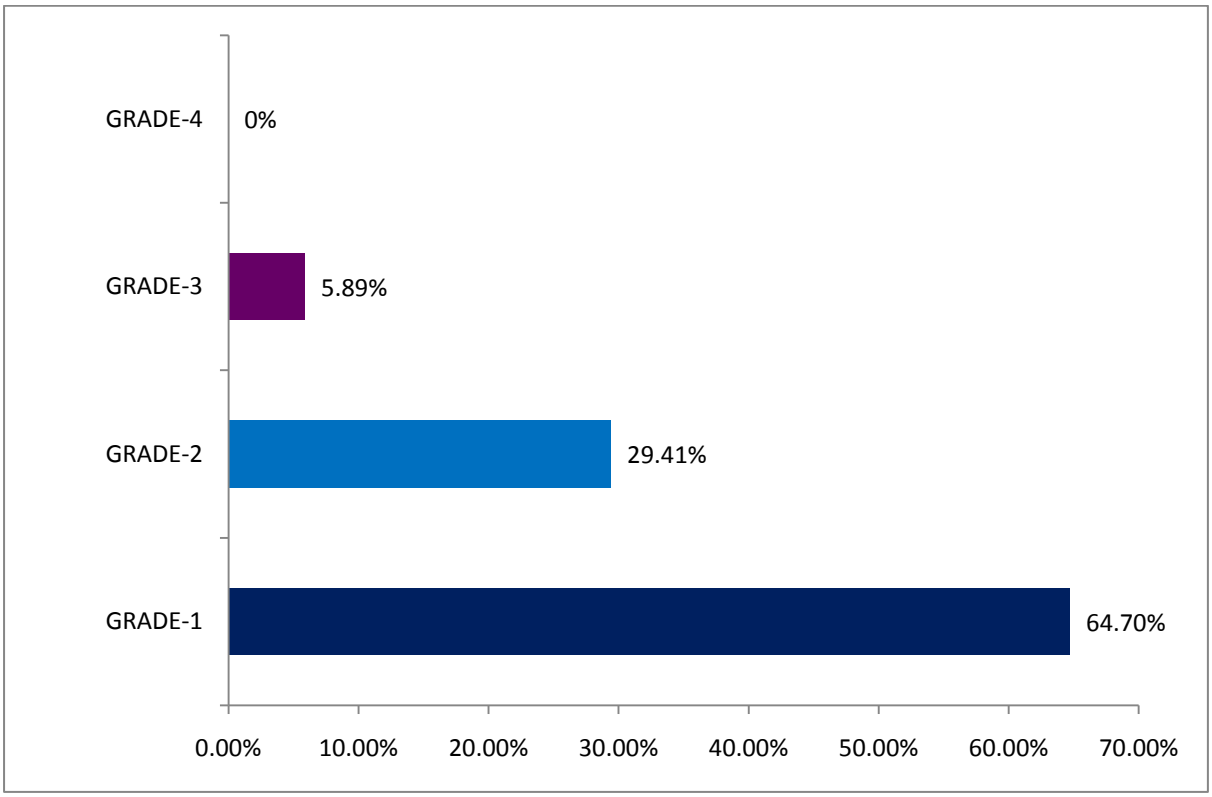
## RETICULIN FIBROSIS AND SECONDARY MYELOFIBROSIS

### TABLE.15

| RETICULIN FIBROSIS | CASES | PERCENTAGE |
|---|---|---|
| GRADE-1 | 11 | 64.70% |
| GRADE-2 | 5 | 29.41% |
| GRADE-3 | 1 | 5.89% |
| GRADE-4 | 0 | 0% |

Most of the cases among secondary myelofibrosis showed grade1 fibrosis.They constitute for 64.70% .Only one case showed grade3 fibrosis.

### CHART - 15



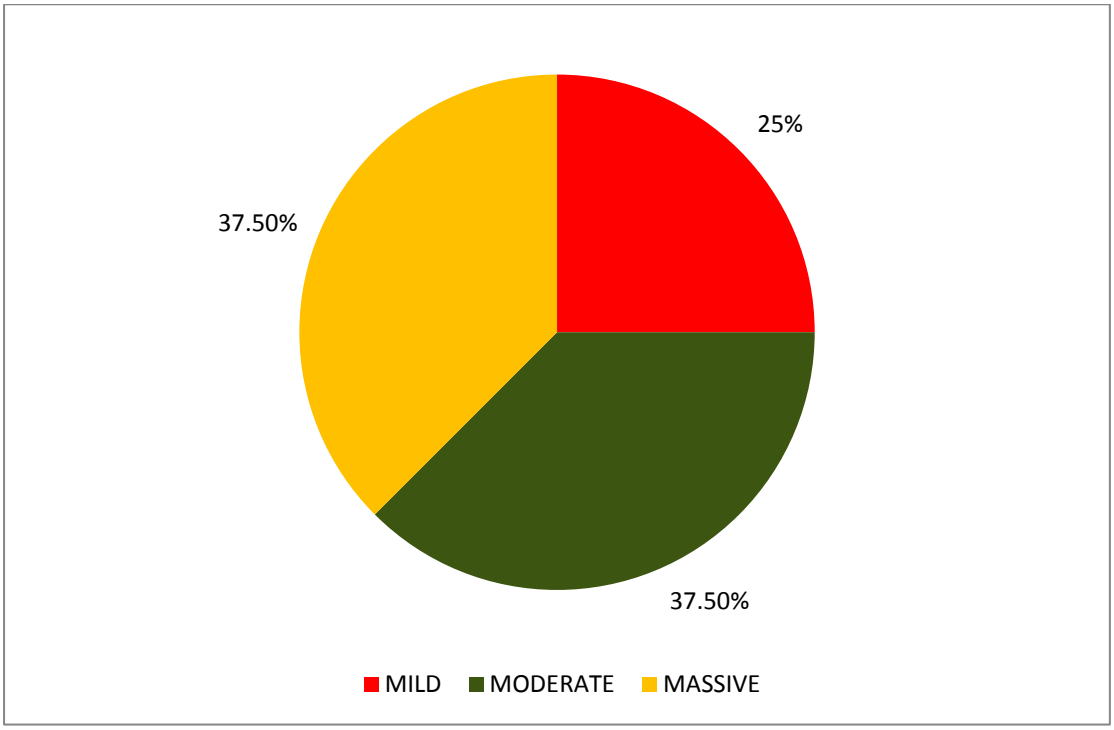
## SPLENOMEGALY AND PRIMARY MYELOFIBROSIS

## TABLE.16

| SPLENOMEGALY | CASES | PERCENTAGE |
|---|---|---|
| MILD | 2 | 25% |
| MODERATE | 3 | 37.5% |
| MASSIVE | 3 | 37.5% |

Splenomegaly was found in all the case.Among the cases studied 3cases(37.5%) had massive splenomegaly.

## CHART- 16



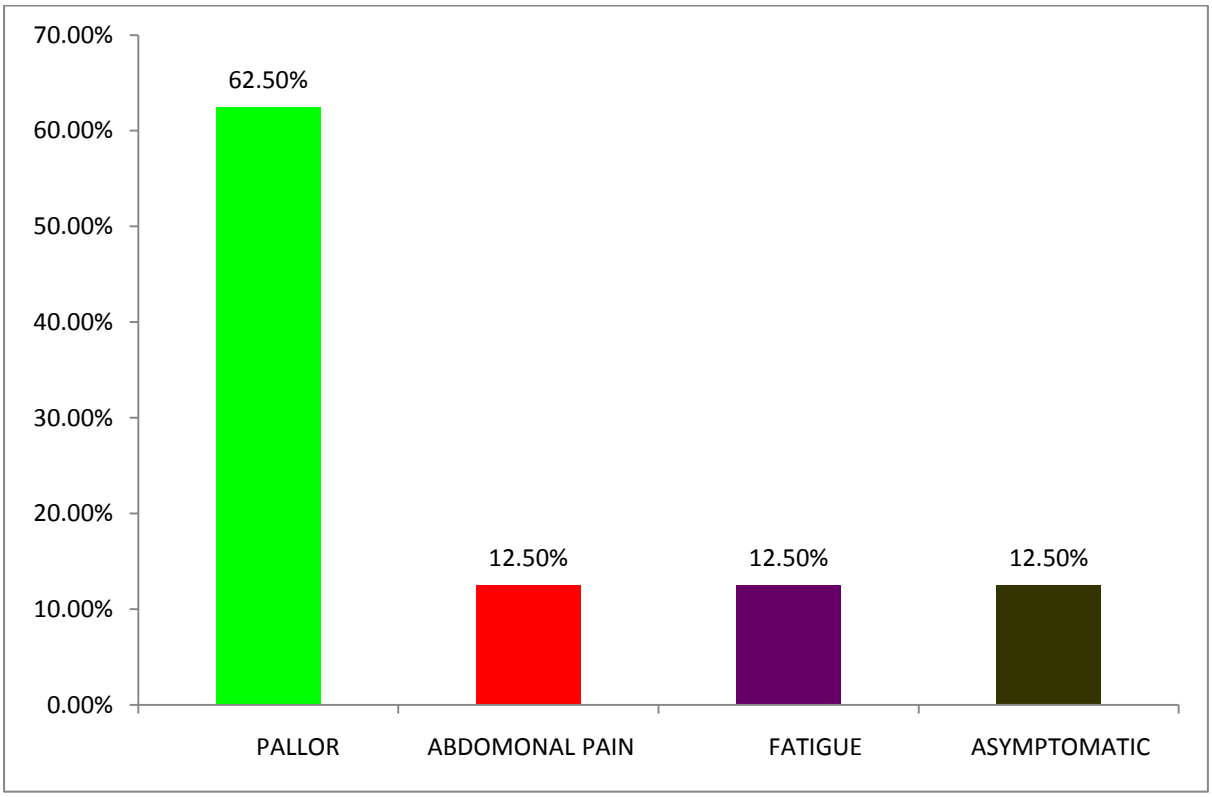
## PRESENTING COMPLAINTS AND MYELOFIBROSIS

**TABLE.17**

| PRESENTING COMPLAINTS | NUMBER OF CASES | PERCENTAGE |
|---|---|---|
| PALLOR | 5 | 62.5% |
| ABDOMONAL PAIN | 1 | 12.5% |
| FATIGUE | 1 | 12.5% |
| ASYMPTOMATIC | 1 | 12.5% |

Idiopathic myelofibrosis presents with wide range of symptoms and signs.Some of the patients are asymptomatic.In this case study 62.5% of patients had pallor.One patient had abdominal pain as a result of splenomegaly.One of the patient presented with no symptoms.

## CHART - 17




Most of the cases presented with the clinical features of anaemia.

## CORRELATION OF MVD,RETICULIN FIBROSIS AND SPLENOMEGALY

### TABLE.18

| S. NO | AGE | SEX | MARROW CELLULARITY | FIBROSIS | SPLENOMEGALY | MVD |
|---|---|---|---|---|---|---|
| 1 | 17yrs | M | HYPERCELLULAR | GRADE-2 | MASSIVE | MVD-3 |
| 2 | 72yrs | M | HYPERCELLULAR | GRADE-2 | MODERATE | MVD-2 |
| 3 | 56yrs | M | HYPERCELLULAR | GRADE-3 | MODERATE | MVD-1 |
| 4 | 52yrs | F | HYPERCELLULAR | GRADE-2 | MASSIVE | MVD-2 |
| 5 | 65yrs | M | HYPERCELLULAR | GRADE-1 | MASSIVE | MVD-1 |
| 6 | 58yrs | F | HYPOCELLULAR | GRADE-3 | MILD | MVD-1 |
| 7 | 51yrs | M | HYPOCELLULAR | GRADE-1 | MODERATE | MVD-1 |
| 8 | 60yrs | M | HYPOCELLULAR | GRADE-3 | MILD | MVD-1 |

Increase in the cellularity is associated with increase in mean vascular density.With increase in the degree of fibrosis the cellularity tends to decrease.Thus there exists inverse relationship between cellularity and fibrosis.There exists no relationship between reticulin fibrosis and splenomegaly.

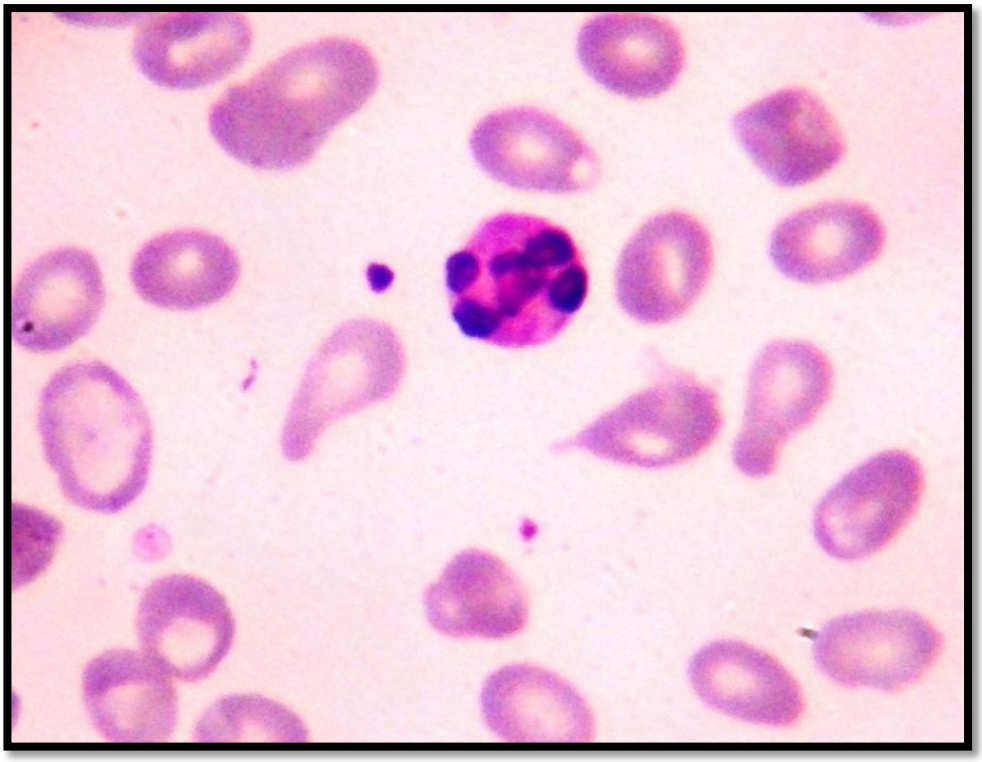


**Fig.1.Smear showing mild anisopoikilocytosis with tear drop cells (1000X)**

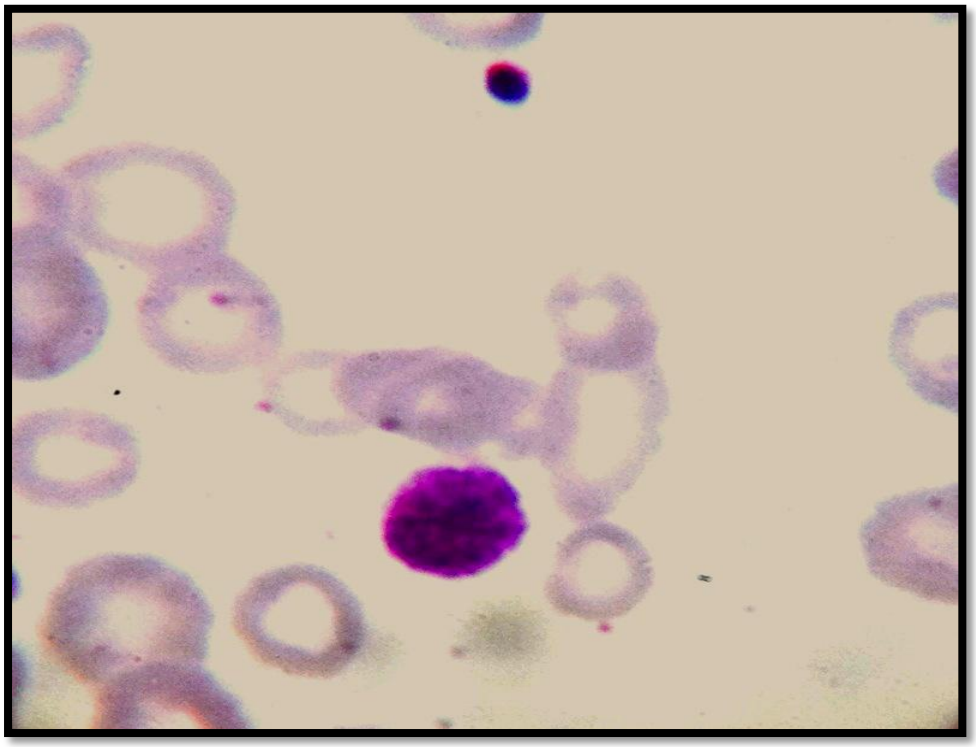


**Fig.2.Smear showing giant platelet (1000X)**

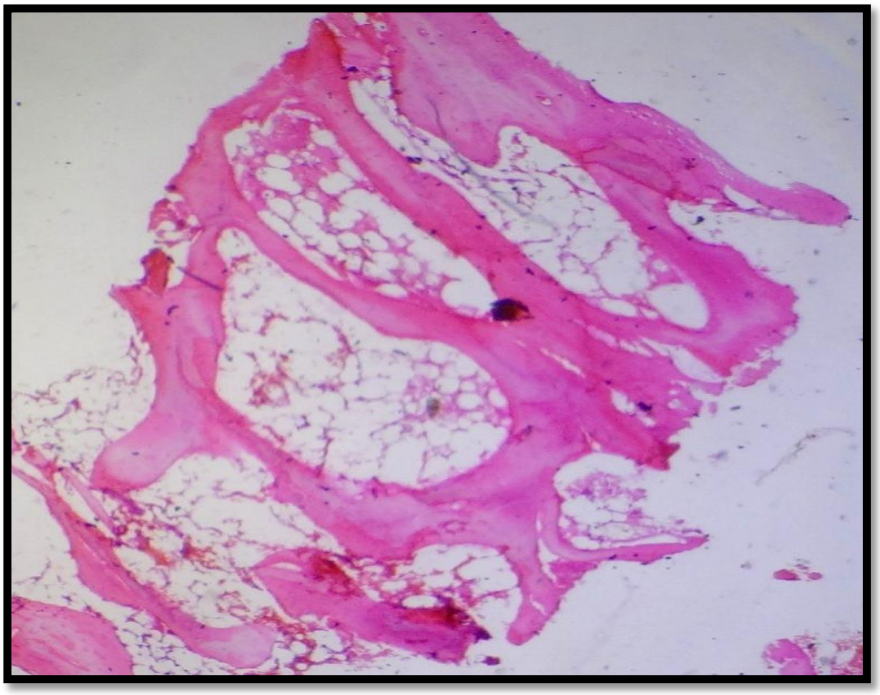


**Fig.3.A Case of aplastic anaemia showing hypoplastic marrow (100X)**

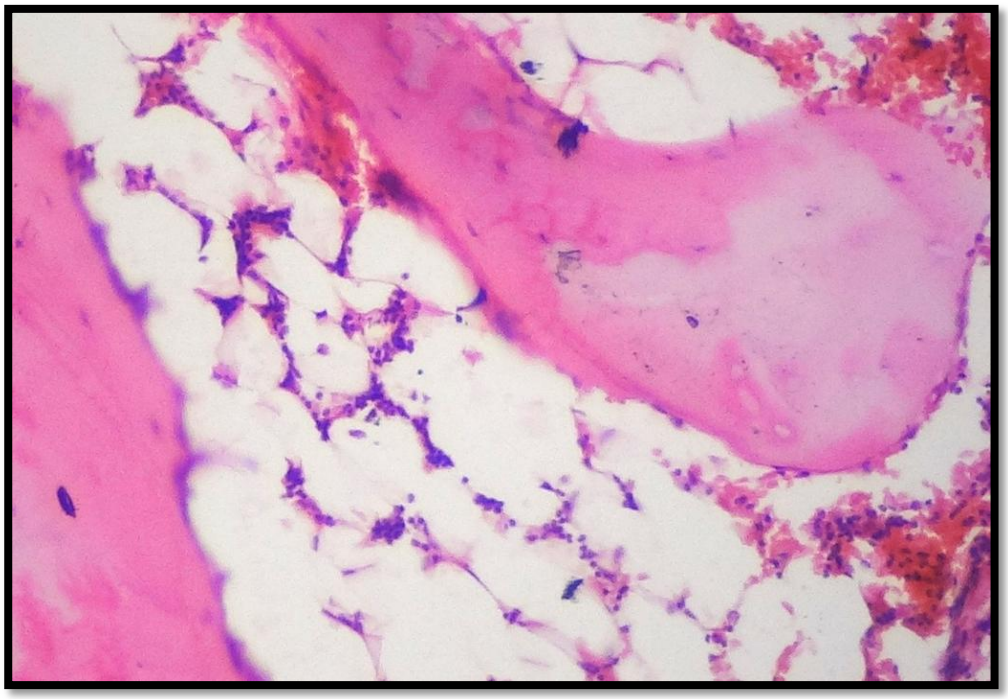


**Fig.4.Bone marrow biopsy showing hypocellular marrow (400X)**

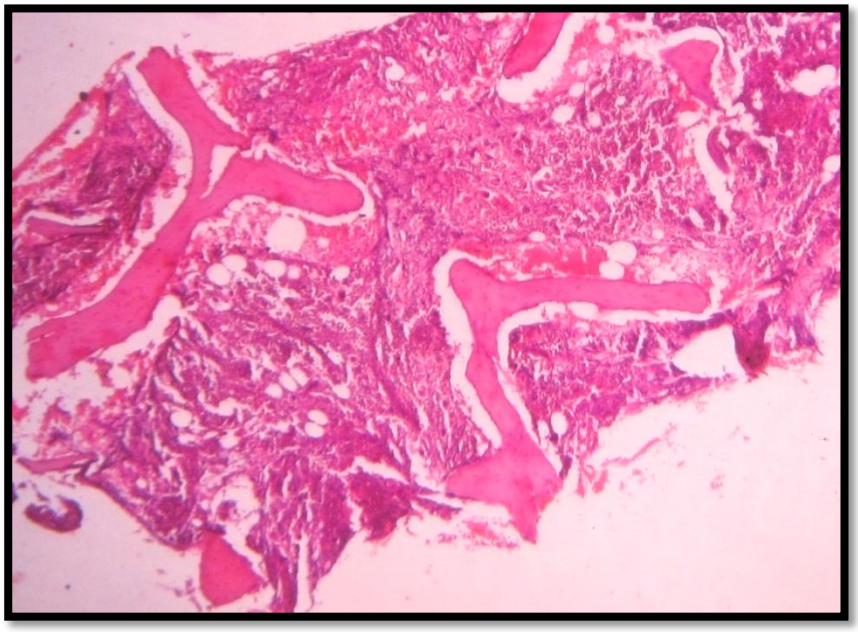


**Fig.5.A case of primary myelofibrosis showing hypercellular marrow (100X)**

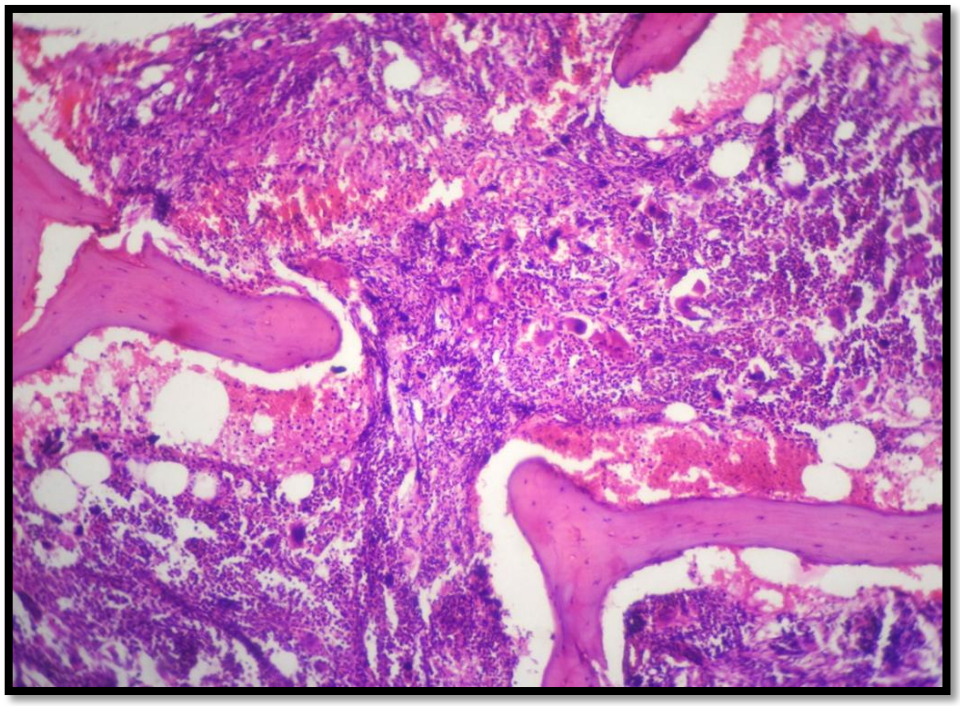


**Fig.6.Hypercellular marrow with trilineage hyperplasia (100X)**

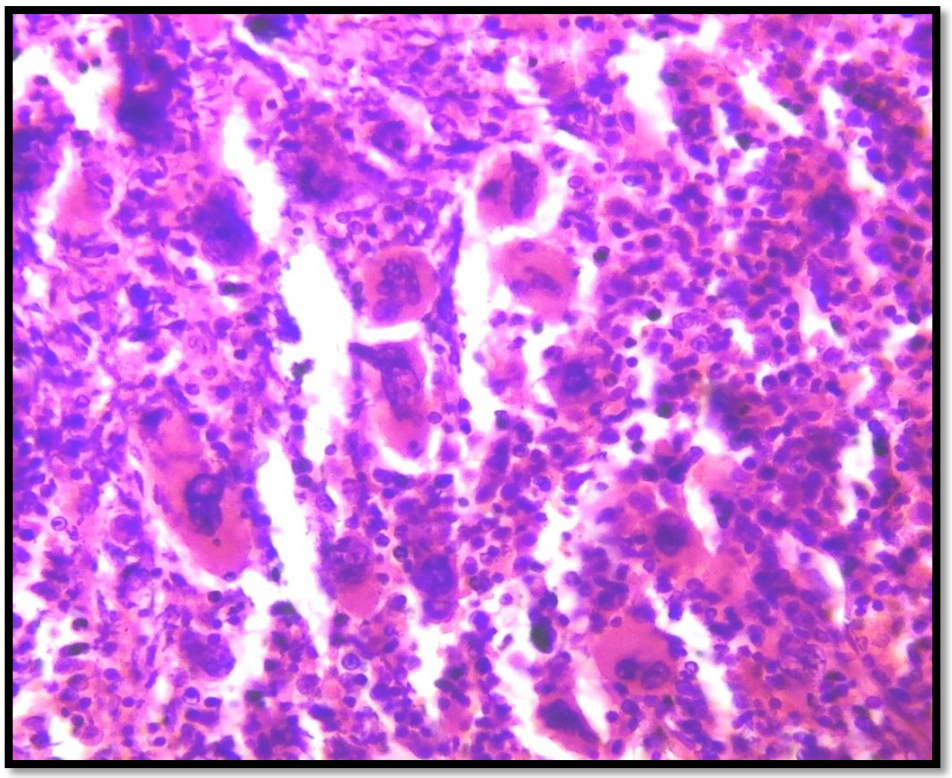


**Fig.7. Megakaryocytic clustering in a case of myelofibrosis (400X)**

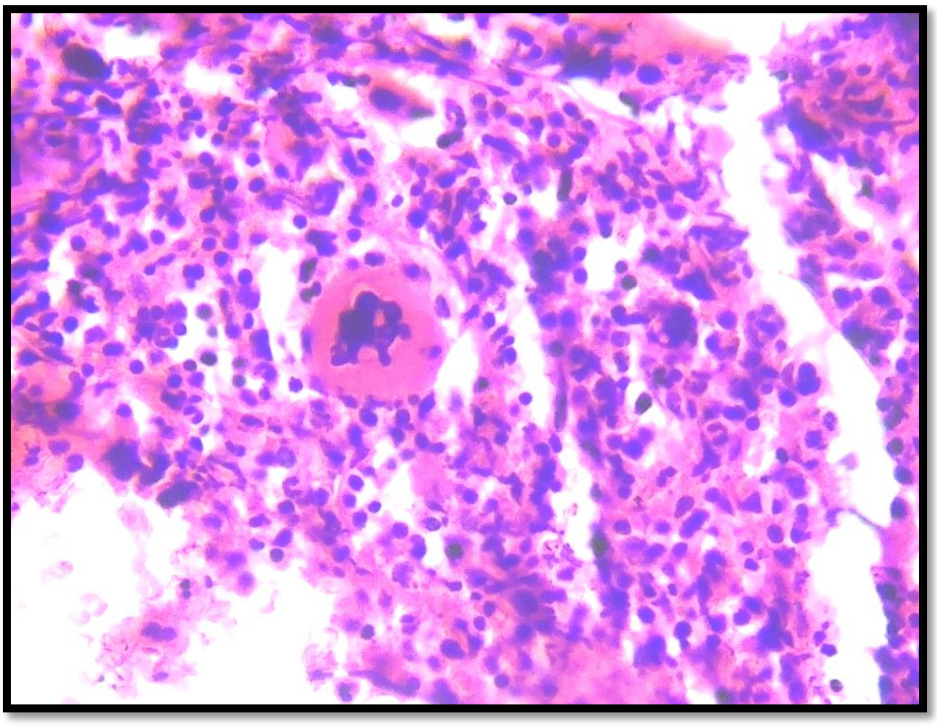


**Fig.8.A megakaryocyte with abnormal lobation (400x)**

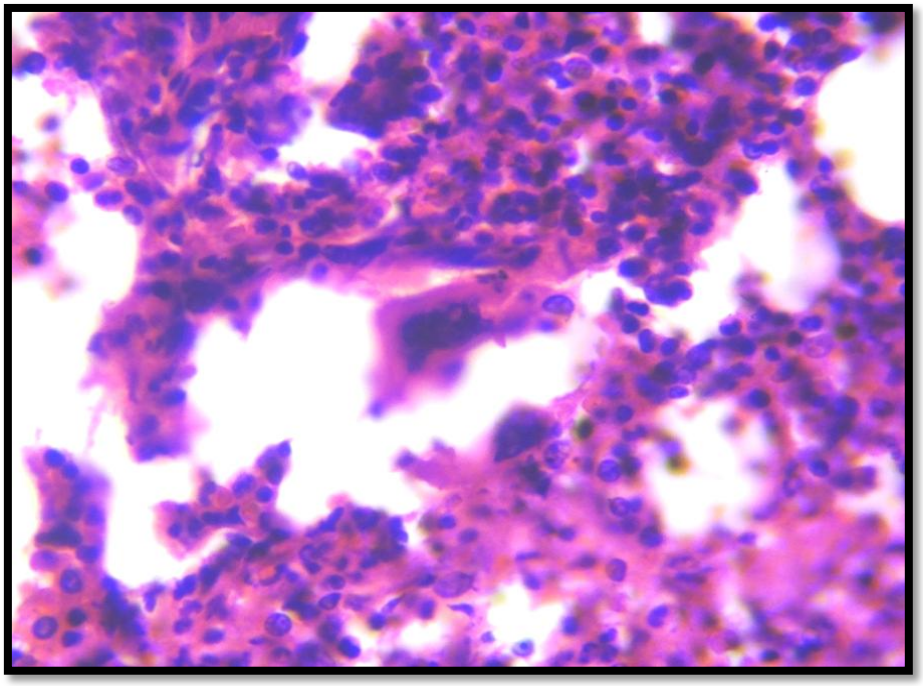


**Fig.9.Intrasinusoidal megakaryopoiesis (400X)**

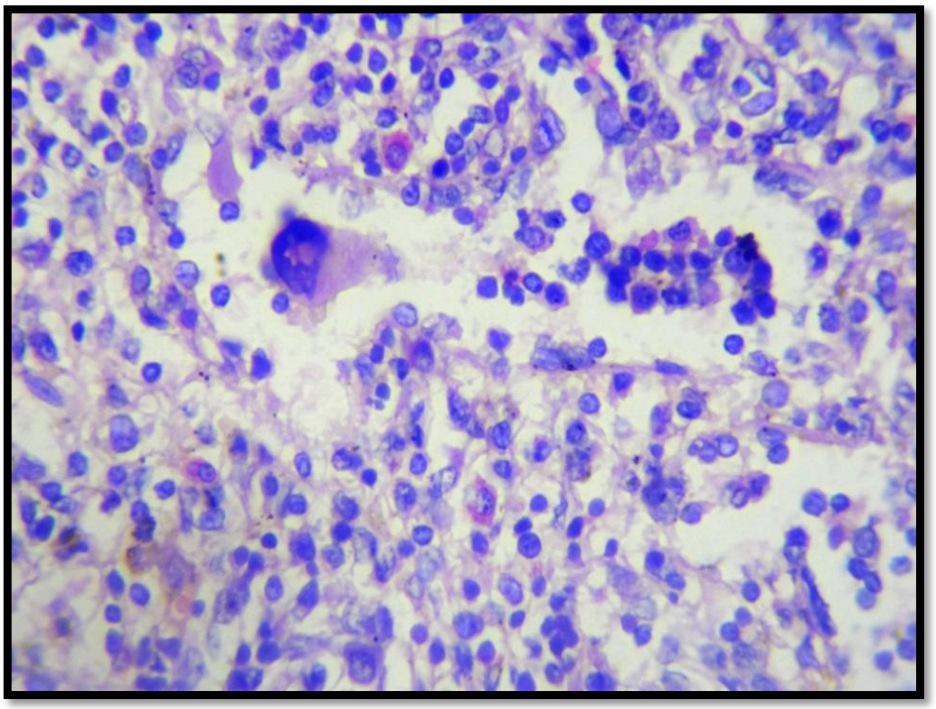


**Fig.10.Intrasinusoidal hematopoiesis in a case of extramedullary hematopoiesis (400X)**

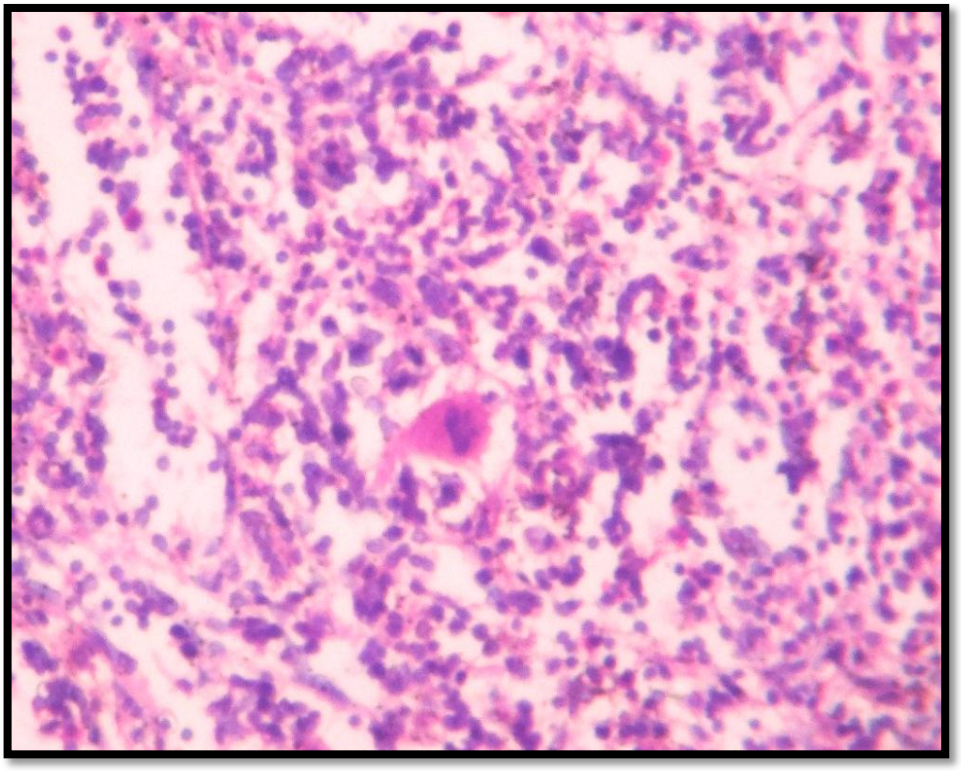


**Fig.11.Extramedullary hematopoiesis in spleen showing a megakaryocyte in the centre (100X)**

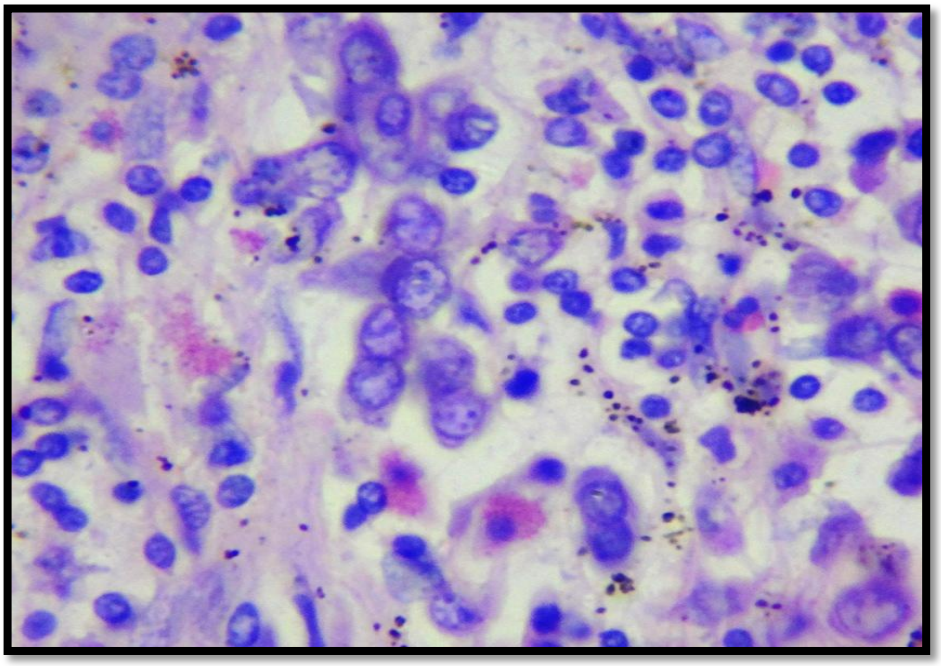


**Fig.12.Cluster of blast like cells in a splenic extramedullary hematopoiesis (400X)**

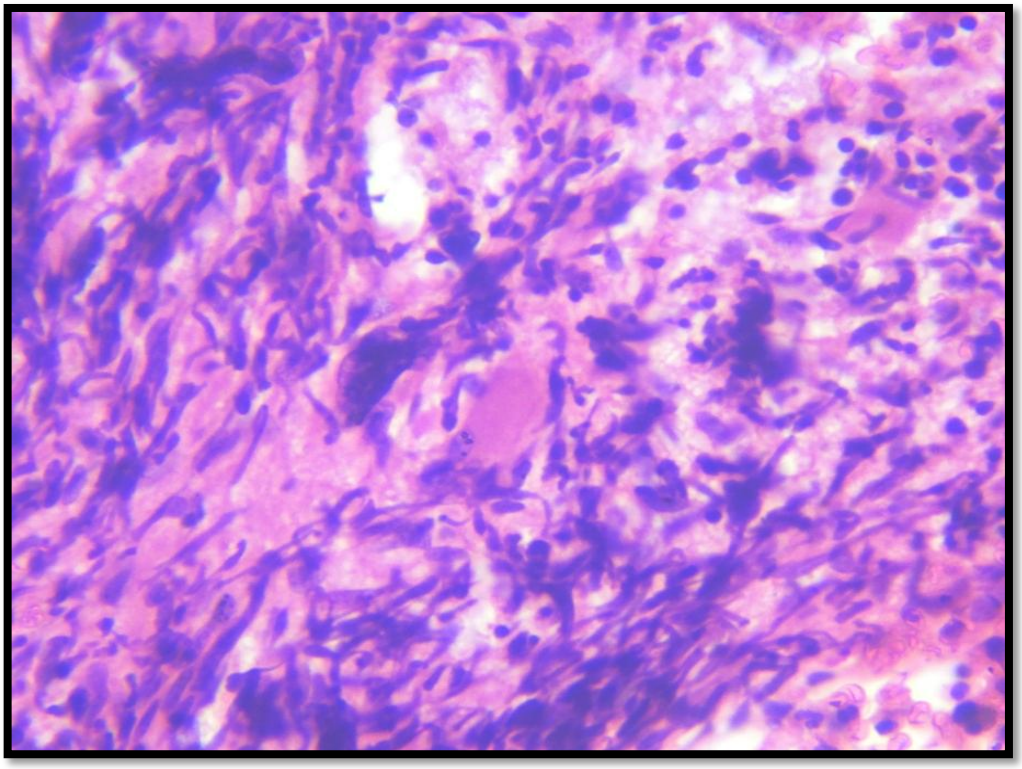


**Fig.13.Early cellular phase of myelofibrosis showing proliferating fibroblasts (400X)**

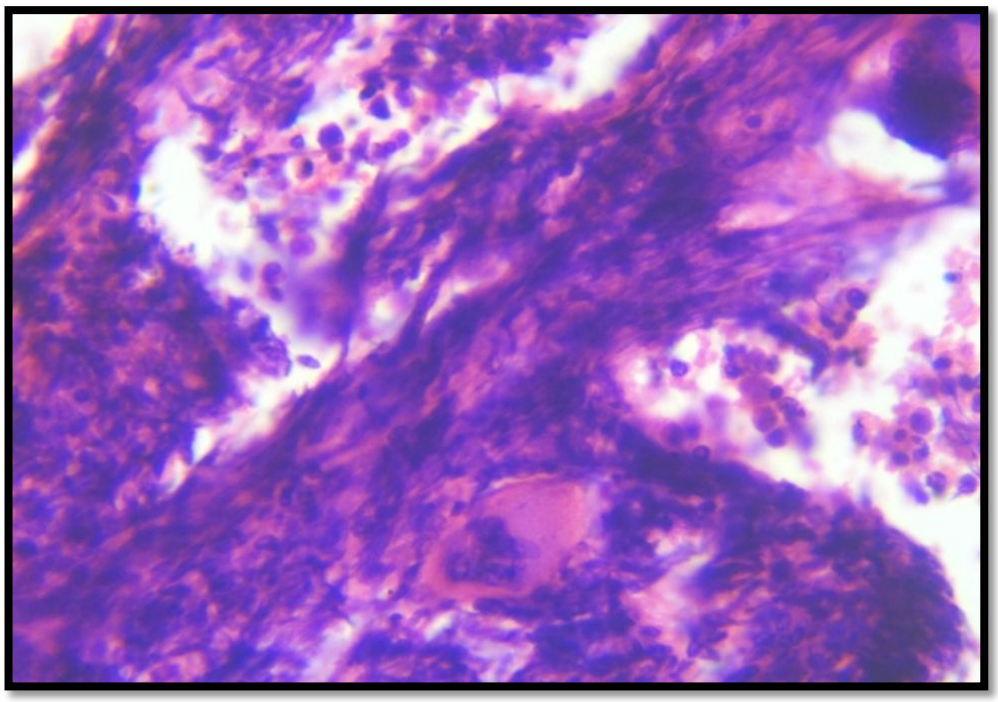


**Fig.14.An entrapped megakaryocyte is seen within coarse fibrosis (400X)**

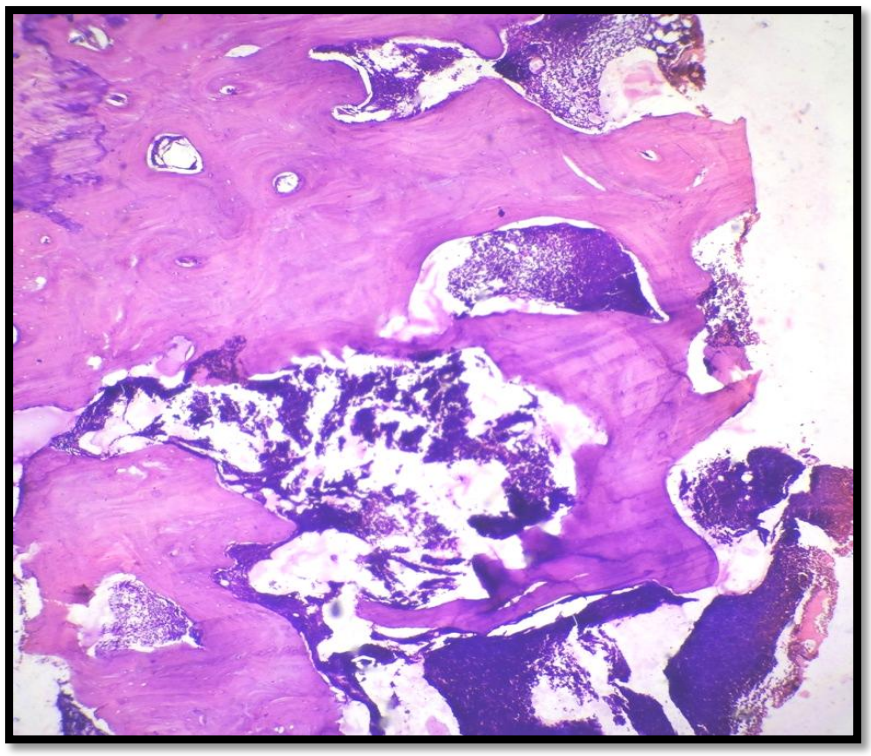


**Fig.15.A case of CLL showing nodular aggregates of monotonous small lymphoid cells (100X)**

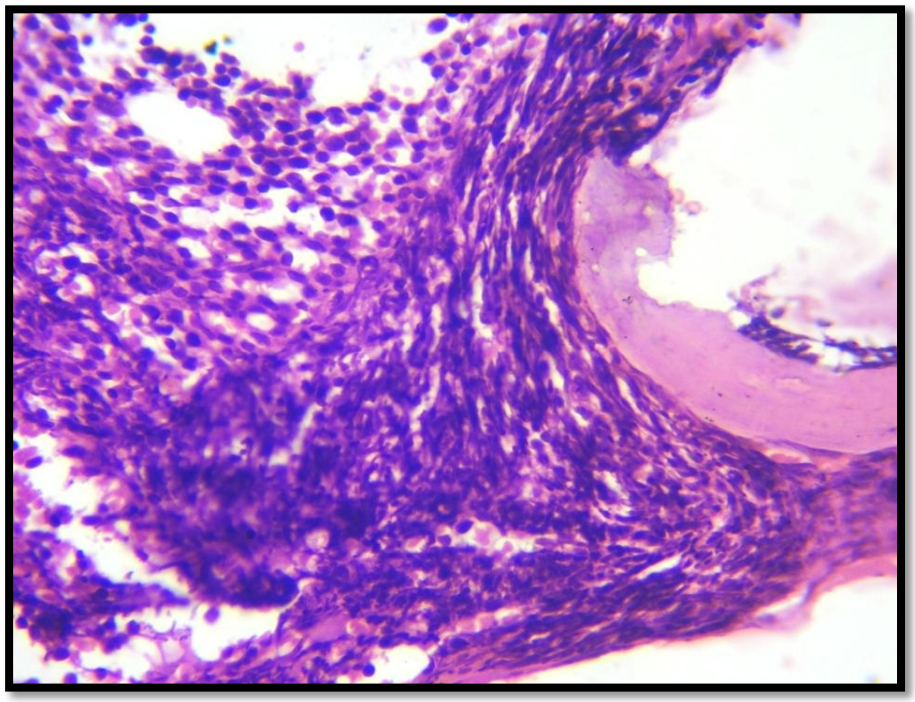


**Fig.16.A case of CLL with areas of fibrosis (400X)**

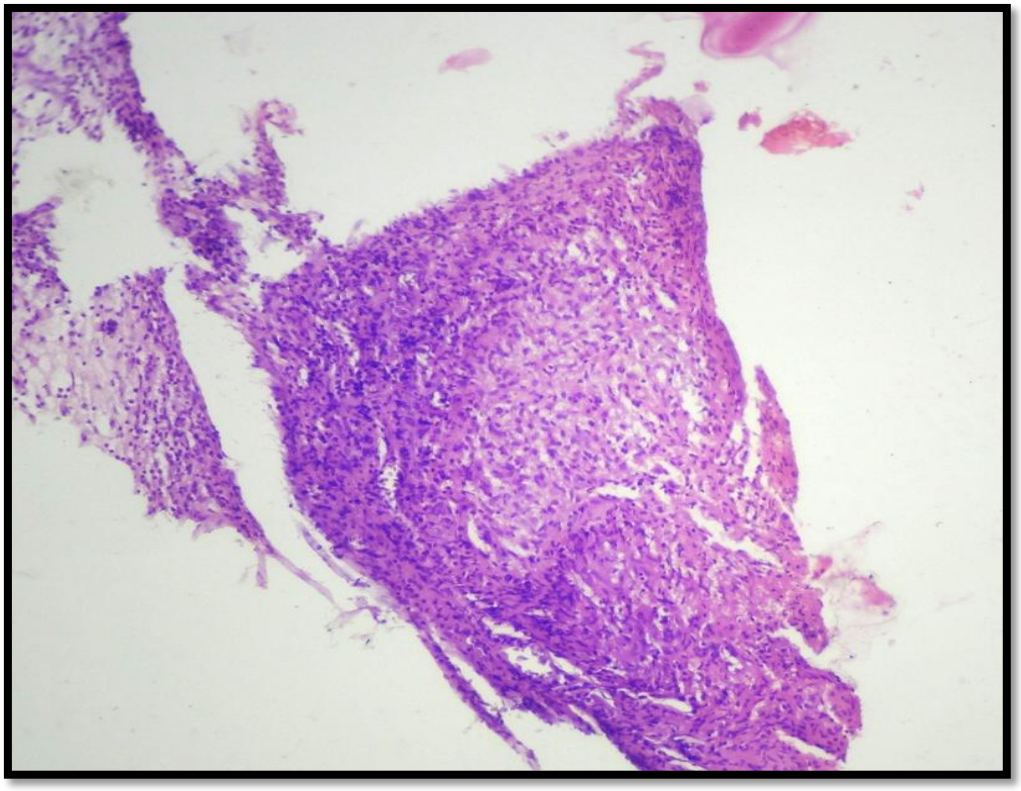


**Fig.17.Epithelioid cell granuloma in a case of disseminated tuberculosis (100X)**

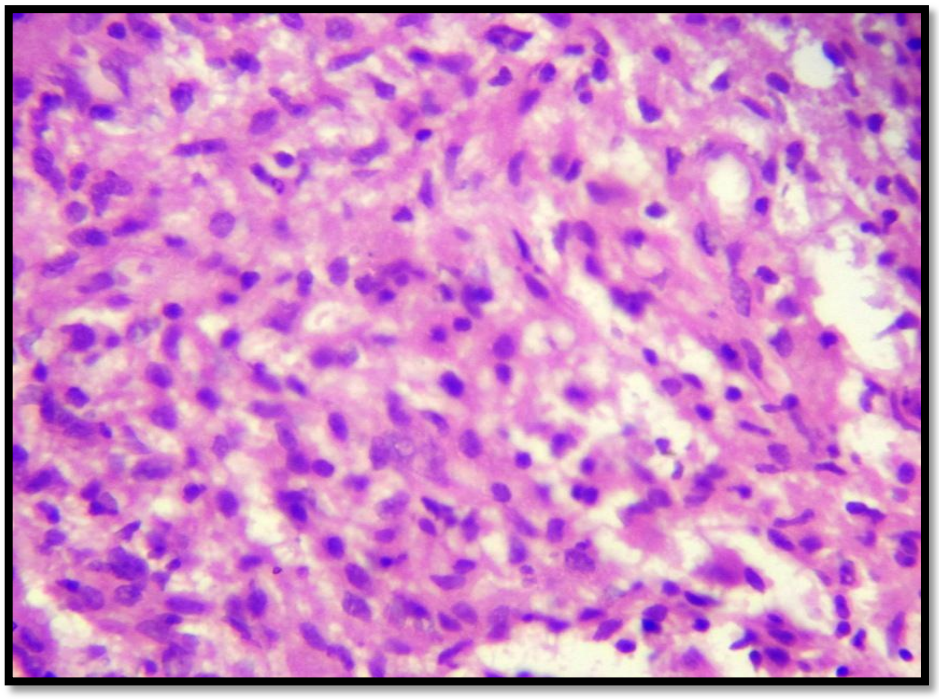


**Fig.18.Epithelioid cells with abundant pale eosinophilic cytoplasm (400X)**

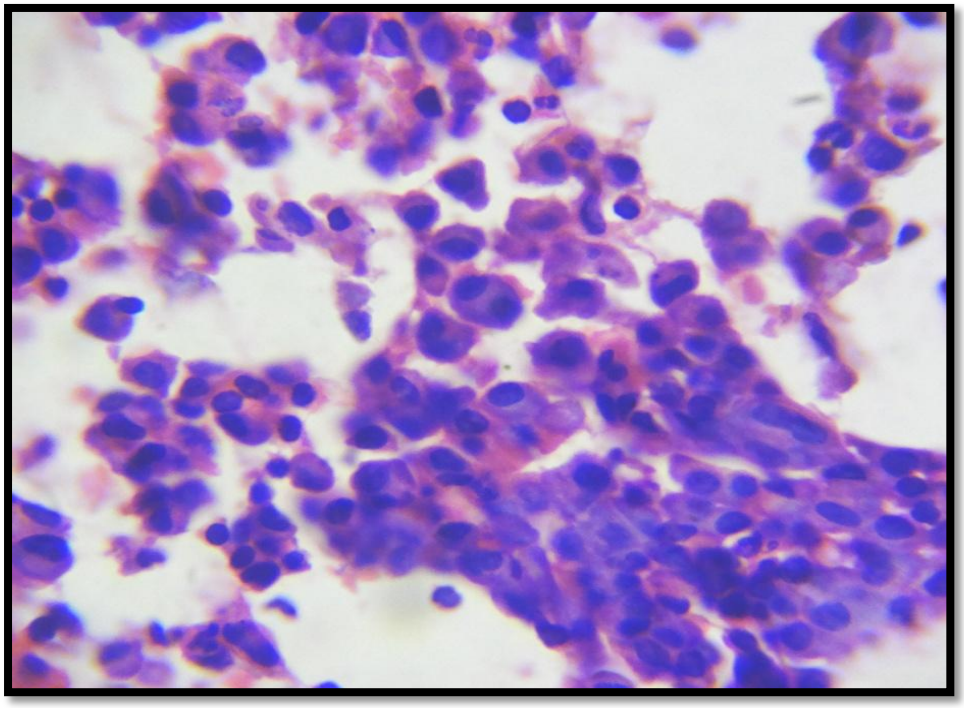


**Fig.19.A case of multiple myeloma with sheet of plasma cells.Few binucleate forms are seen (400X)**

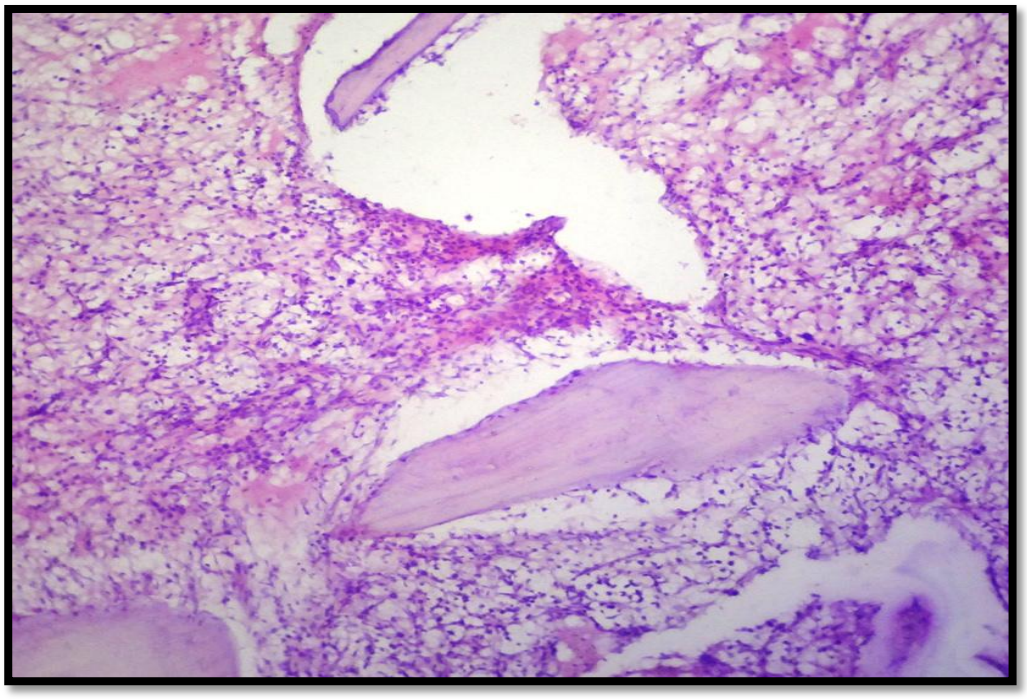


**Fig.20.A case of SCC (Tonsil) showing radiation induced secondary myelofibrosis (100X)**

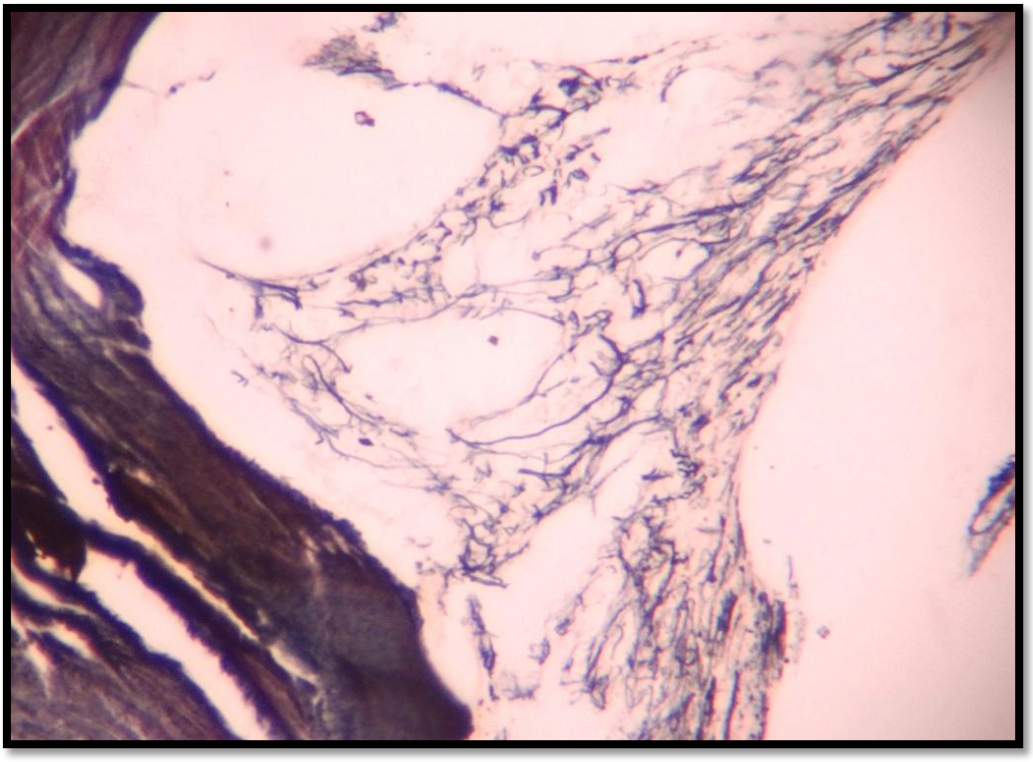


**Fig.21.Grade 1 reticulin fibrosis (100X)**

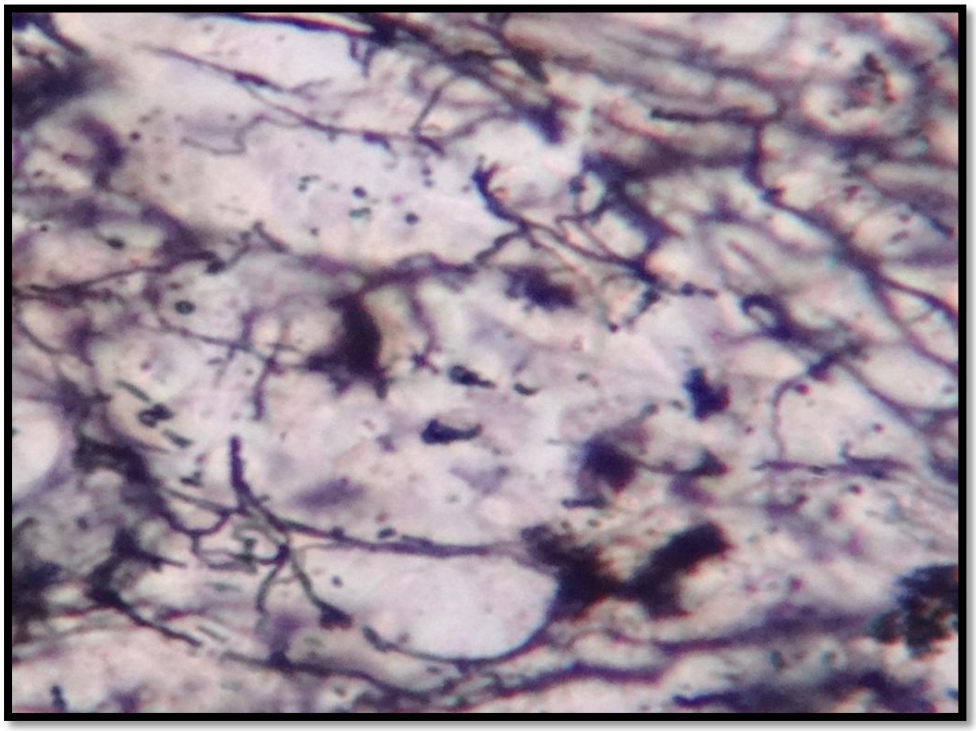


**Fig.22.Fine scattered reticulin fibres (400X)**

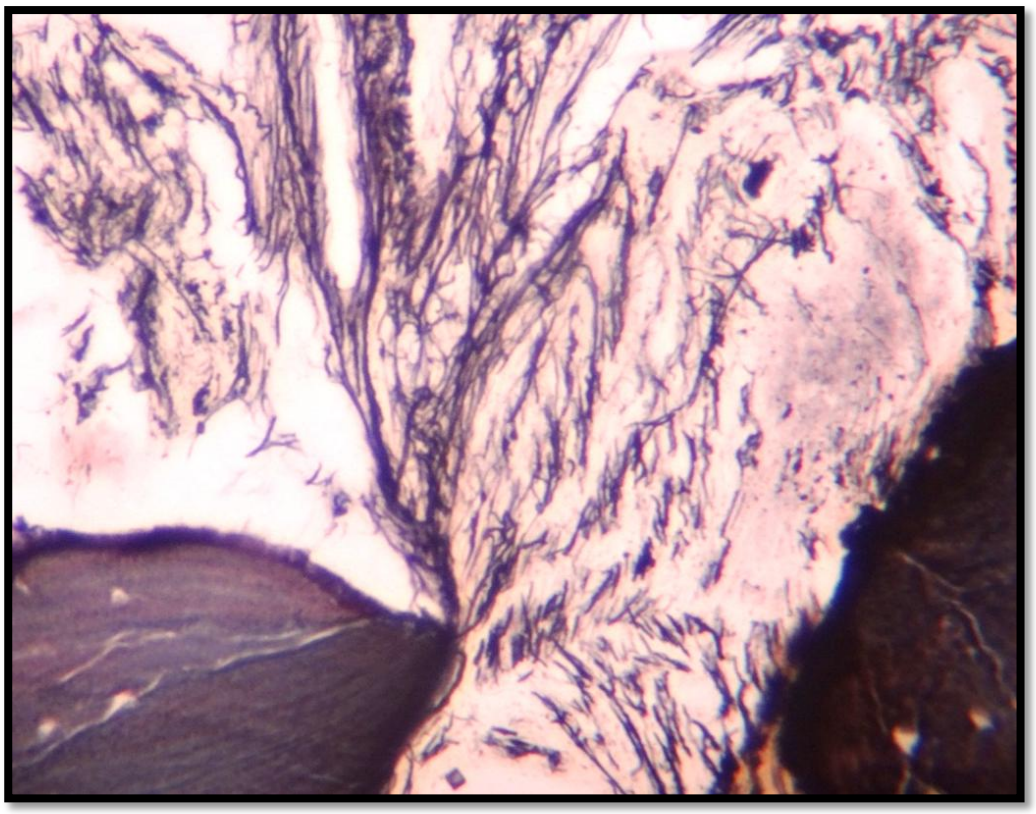


**Fig.23.Grade 2 reticulin fibrosis (100X)**

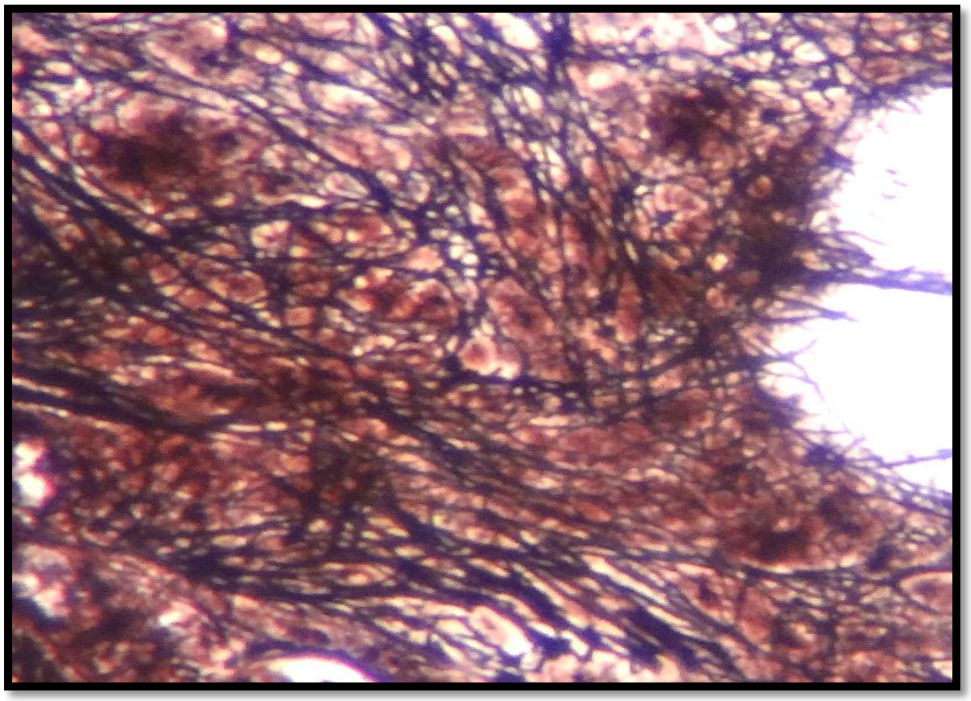


**Fig.24.Fine reticulin fibres with intersection (100X)**

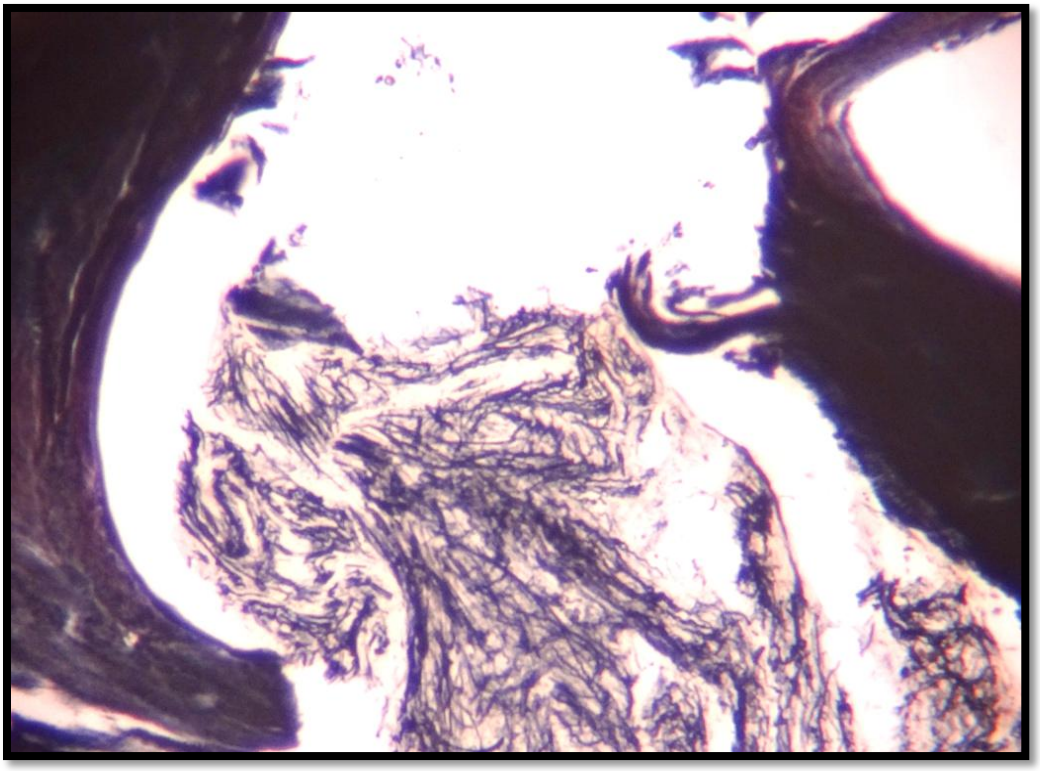


**Fig.25.Grade3 reticulin fibrosis (100X)**

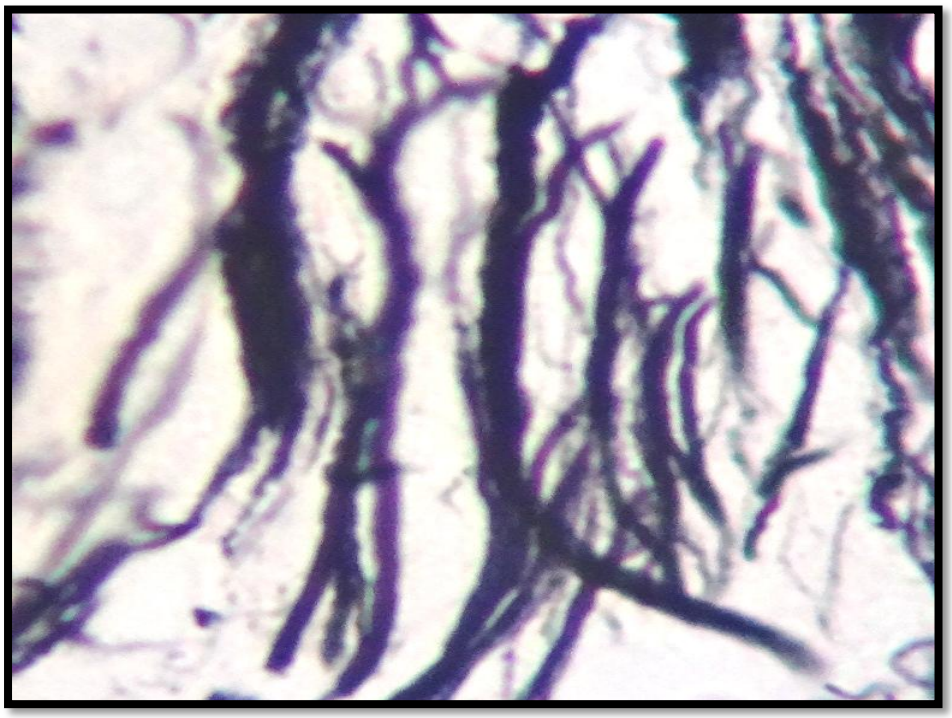


**Fig.26.Coarse reticulin fibres (400X)**

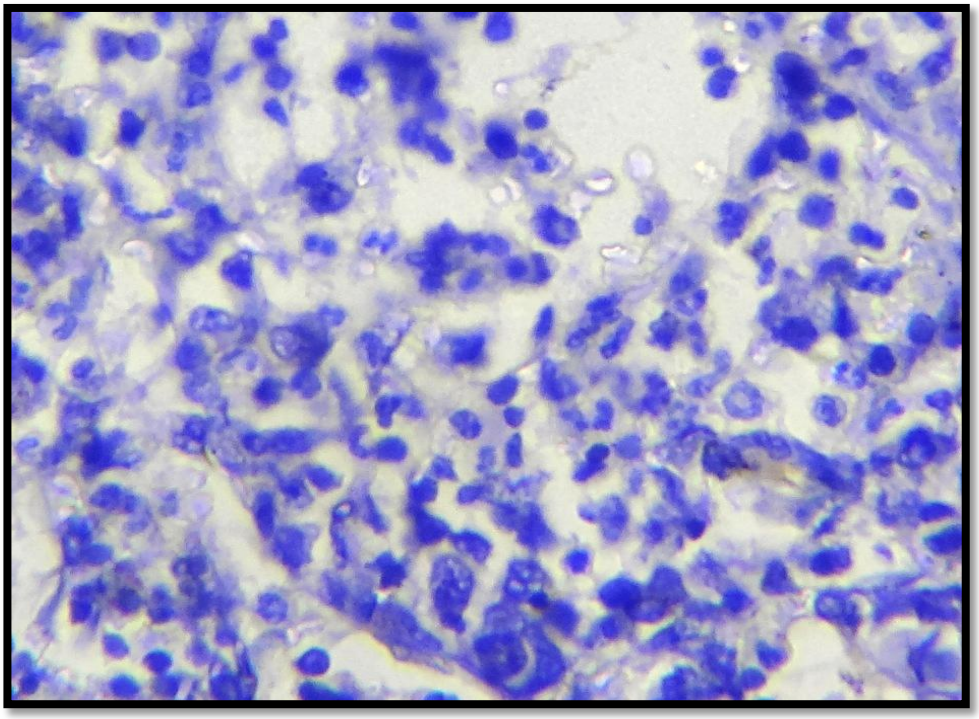


**Fig.27.No increase in mean vascular density (400X)**

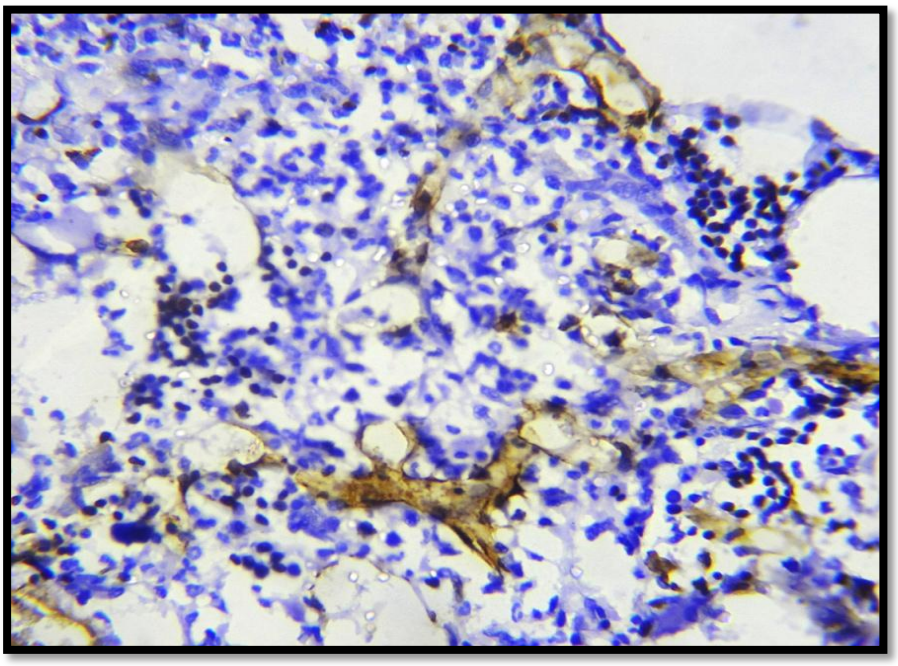


**Fig.28.Mean vascular density-Grade1 (400X)**

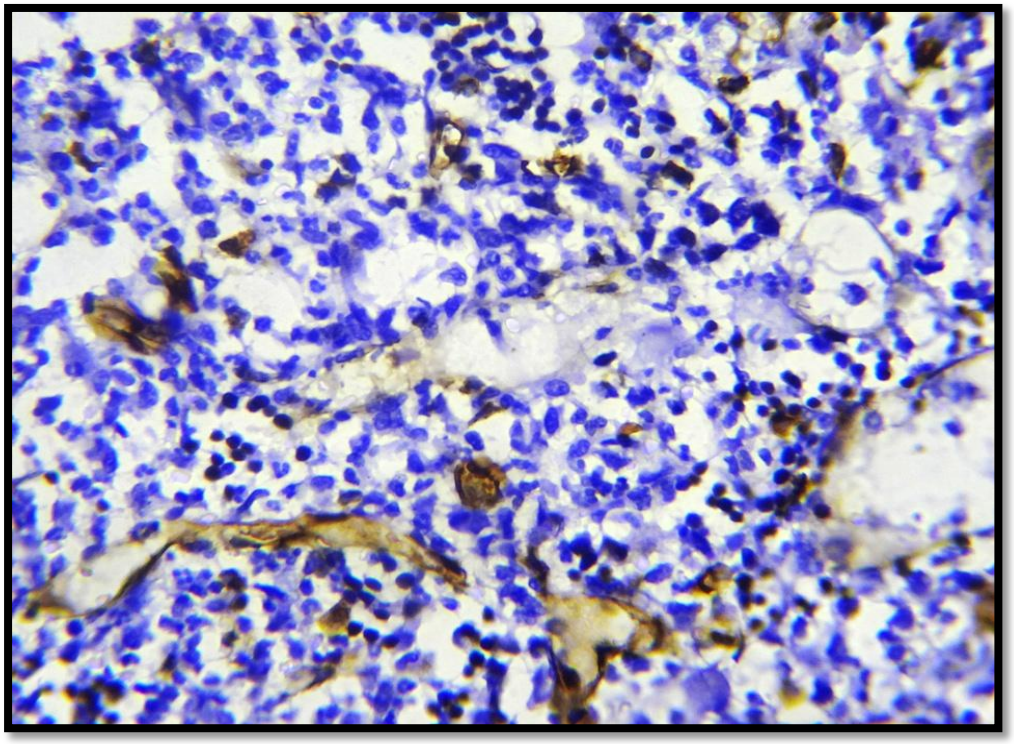


**Fig.29.Mean vascular density-Grade2 (400X)**

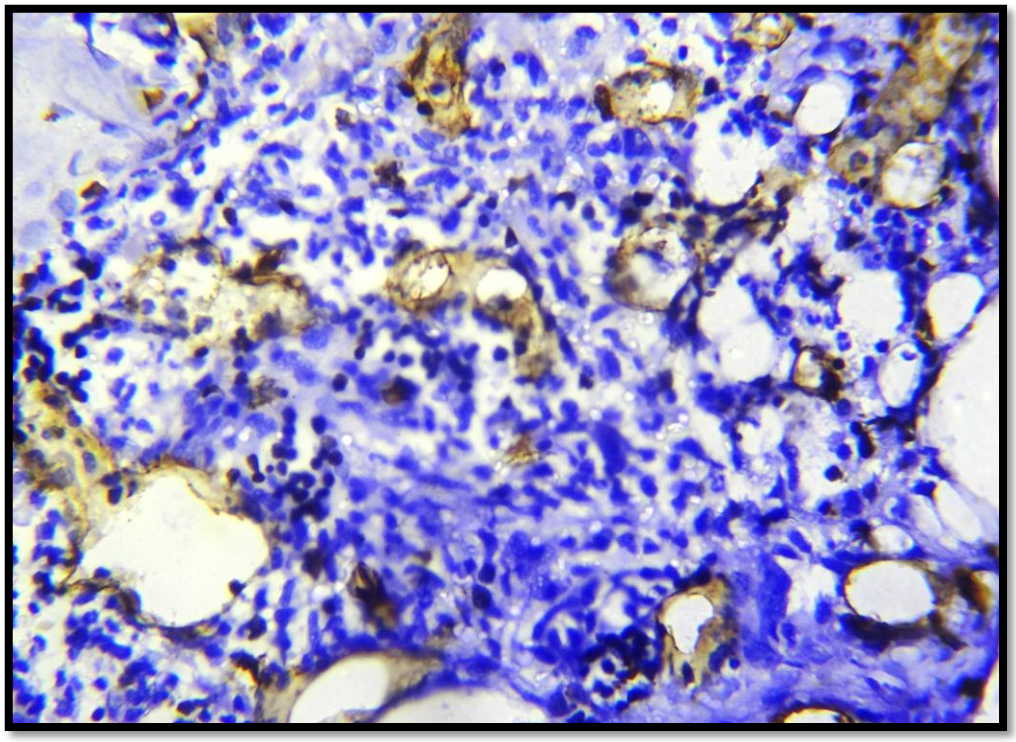


**Fig.30.Mean vascular density-Grade3 (400X)**

## DISCUSSION

Idiopathic myelofibrosis is a clonal myeloproliferative neoplasm in which the proliferation of multiple cell lineages is accompanied by progressive bone marrow fibrosis.

### AGE AND SEX DISTRIBUTION

According to Barosi.G et al and ozen.s et al idiopathic myelofibrosis characteristically occurs after 50 years of age and the mean age at diagnosis is 65years. In this study most of cases of primary myelofibrosis were over 50years and the mean age group at diagnosis was 54. This is comparable with other studies.

According to Cervantes et al (1998) myelofibrosis can occur from the neonatal period to the ninth decade of life. In this study lowest age group affected was 17years and highest age group was 72 years.

According to okamura.T et al (2001) myelofibrosis, in adults occurs with about equal frequency in men and women. In this study male to female ratio is 3:1(male preponderance). This may be probably due to low number of cases involved in the study.

In addition to this in this study ratio of primary and secondary myelofibrosis is found to be 1:2(1:2.125) which indicates that secondary myelofibrosis is more common than primary.Male to female ratio in secondary

myelofibrosis,according to this study is 1:1.4 (with slight female preponderance).

## SYMPTOMS AND CLINICAL FEATURES

According to Varki et al 71% of the cases of myelofibrosis present with the complaints of fatiguability and 21% cases presents with no symptoms (asymptomatic).

According to Silverstein et al 60% of patients present with pallor. From above mentioned studies it is clear that maximum percentage of cases present with clinical symptoms of anaemia. In this study 75% of cases presented with clinical symptoms and signs related to anaemia.

## HEMATOLOGICAL PROFILE AND CELL MORPHOLOGY

According to Tefferi A et al, 60% of the cases have their haemoglobin level dropped below 10g /dl. In this study, all the cases of primary myelofibrosis (100%) had their haemoglobin level below 10gm%. However, only 53% of cases of among secondary myelofibrosis had their Hb% below 10gm/dl. Although all the cases of primary myelofibrosis presented had their haemoglobin percentage below 10g/dl,it is often difficult to estimate the degree of anaemia using haemoglobin (or) Hematocrit, as most of the individuals with increased spleen size have expanded plasma volume resulting in anaemia.

According to Barosi et al the most common type of anaemia among the patients of idiopathic myelofibrosis is normocytic normochromic anaemia. Most

common type of anaemia among the cases in this study is found to be normochromic and normocytic with dacryocytes in 75% of the patients of primary myelofibrosis and anisopoikilocytosis in 87.5% of the patients.None of the patients presented with haemolytic anaemia. This shows that none of the cases had hypersplenism (or) anti-erythrocyte autoantibodies.

According to Tefferi et al 13% to 25% of patients present with leucopenia and leucocytosis occurs in one third of the patients. In this study 6 out of 8 patients presented with leucopenia while two of the patients had normal total leukocyte count. No patient presented with leucocytosis. This may be probably due to the fact that, most of the patients presenting in late phase of disease.

In Mayo clinic series of 169 patients, platelet count of less than 1,00,000/$mm^3$ was found in 31% of the patients. In this study all the patients(100%) presented with thrombocytopenia. Giant platelets were observed in 2 out of 8 cases. 62.5%(5 out of 8 cases) had their platelet count between 60,000/$mm^3$ and 1,00,000/$mm^3$.

**FIBROSIS IN DISORDERS OTHER THAN PRIMARY MYELOFIBROSIS**

According to Najeany et al fibrosis can accompany various disorder like polycythemia vera and chronic myeloid leukemia.In this case study we had

fibrosis accompanying 2 cases of CML (2 out of 17).It constituted for 11% of total number of cases. In one case it was present at the time of diagnosis. In other case, it was found 8 months after initial diagnosis.

Pagliuca and co-workers described a variant called MDS with fibrosis. In this case study, we had one case of MDS with fibrosis((1 out of 17)-5%). The patient presented with pancytopenia. The marrow showed low cellularity with fibrosis. The case was finally diagnosed as MDS with transformation into AML by flow cytometry.

According to Crail et al disseminated tuberculosis and histoplasmosis are the two important infectious diseases resulting in secondary myelofibrosis.

According to Kiely et al and Kiang et al metastatic deposits from stomach,breast and prostate commonly results in secondary myelofibrosis.

In this case study we had one case of CLL with fibrosis, 0ne(1) case of TB with fibrosis, two(2) cases of lymphoma with fibrosis and two(2) cases of metastatic carcinomatous deposit with fibrosis.

Three(3) cases of Acute myeloblastic Leukemia showed fibrosis in bone marrow trephine biopsy.

One(1) case developed secondary myelofibrosis after radiotherapy. She had a initial diagnosis of Squamous cell carcinoma tonsil and presented with secondary myelofibrosis one year after radiotherapy.

The results of this study were comparable to studies done by others.

## SPLENOMEGALY

Splenomegaly is a constant and important feature of myelofibrosis (IMF). According to Visani et al 99% of patients with IMF had splenomegaly.According to Varki et al and Silverstein et al 90% of patients had splenomegaly. In this study (7 out of 8) 87.5% of patients had splenomegaly. Among these patients, one underwent splenectomy due to complicating symptoms of splenomegaly. Histopathological examination of splenectomy specimen revealed extramedullary hematopoiesis.

Increase in the spleen size is irrespective of degree of fibrosis.Some of the patient with mild degree of fibrosis had more splenomegaly and some patients with high degree of reticulin fibrosis had mild splenomegaly.This reflects the fact that splenomegaly does not depends upon the degree of reticulin fibrosis. This is comparable to study results given by BC Wolf and RS Nieman.

## BONE MARROW CELLULARITY AND FIBROSIS

In Wolf and Nieman series of case study bone marrow sections revealed a wide range of morphological changes. The degree of stromal proliferation was extremely variable. Some biopsies out of 35 patients in his study showed 100% cellularity.The myeloid,erythroid and megakaryocytes series were all increased.

The cellular biopsies showed only slight increase in reticulin fibrosis. Majority of the cases,showed only reticulin fibrosis with two cases out of 35 patients showing collagenous fibrosis.

In Wolf and Nieman case study they have observed three constant findings in cases of IMF. They are patchy stromal fibrosis,increased number of megakaryocytes with megakaryocyte clustering. The megakaryocyte clustering appeared to be most numerous in areas of extensive fibrosis.Third feature was the distended sinusoids with frequent intrasinusoidal hematopoiesis.

In this study out of 25 cases 8 cases showed features of primary myelofibrosis. Among 8 cases, all the cases showed stromal proliferation and reticulin fibrosis.Stromal proliferation and reticulin fibrosis were patchy in the hypercellular marrow and diffuse in case of hypocellular marrow.Hence it can be considered that increase in reticulin fibrosis is associated with decrease in the bone marrow cellularity. This is similar to results given by Wolf and Nieman.

In this study also megakaryocytic proliferation and megakaryocytic clustering were more conspicuous in cases of prefibrotic myelofibrosis. Out of 8 cases, 3 cases showed megakaryocytic clustering and 5 cases showed dysplastic megakaryocytes. Intrasinusoidal hematopoiesis was observed in 2 cases. All the 3 cases which showed megakaryocytic clustering were the cases of prefibrotic myelofibrosis.

According to Wolf and Nieman,intrasinusoidal hematopoiesis was apparent in the hypocellular marrows. But in this study intrasinusoidal hematopoiesis was readily observed in cellular marrows with accompanying fibrosis.

**BONE MARROW MORPHOMETRY AND MEAN VASCULAR DENSITY**

According to Mesa et al 2000[38] and panteli et al 2005[39] angiogenesis is more evident in Primary myelofibrosis. In their study patients with PMF were found to have significantly higher values of MVD than those with other disorders. In this study also there is a substantial increase in the mean vascular density in case of IMF when compared to other cases. Although only some cases (3 out of 8 cases) showed intense increase in the mean vascular density. This may be due to decrease in the bone marrow cellularity with accompanying increase in the reticulin fibrosis.

According to Mesa et al 2000[38] there is increase in marrow angiogenesis, with increase in bone marrow cellularity and megakaryocyte clustering and were independent of reticulin fibrosis. There is a inverse relationship between bone marrow cellularity and fibrosis.

In this study, 5 patients had hypercellular marrow.Among 5 patients, 3 patients had substantial increase in the mean vascular density. Other two patients had MVD which were comparable to normal controls. The cases with

hypocellular marrow does not show significant increase in marrow mean vascular density.

According to this study,all the patients who had increase in the mean vascular density also had increased spleen size. Hence it may be considered that vascular proliferation may be a mother event in causing splenomegaly and disease progression.

However some patients had increase in the spleen size without increase in the Mean vascular density. In such cases, increase in spleen size may be attributed to the increased disease duration.

## SUMMARY

The study included total of 25 cases. Among 25 cases,17 cases were cases of secondary myelofibrosis.

- Most of the cases of secondary myelofibrosis were hematological malignancies including leukemia and lymphoma.
- Disseminated tuberculosis was the most common infectious disorder resulting in secondary myelofibrosis.
- Primary myelofibrosis constitutes for 38% of total cases.
- IMF was mostly diagnosed in $5^{th}$ decade and $6^{th}$ decade with mean age of 54 years in this study.
- All the patients of IMF presented with Anaemia and thrombocytopenia.75% of cases presented with pancytopenia.
- Megakaryocyte clustering and dysplastic megakaryocytes were more conspicuous in prefibrotic stage of myelofibrosis.
- Almost all the cases of primary myelofibrosis showed anisocytosis and tear drop cells.
- Most common type of anaemia in IMF is normocytic normochromic.
- Most of the cases of IMF had moderate to severe splenomegaly.

- Increase in bone marrow cellularity was associated with increase in MVD.
- Increase in fibrosis is associated with decrease in cellularity.

# CONCLUSION

Idiopathic myelofibrosis is a rare myeloproliferative disorder characterised by increase in cellularity, bone marrow reticulin fibrosis and increased megakaryocytic clustering. Although reticulin fibrosis is a characteristic feature of Idiopathic myelofibrosis, it may be less conspicuous in early prefibrotic stage.

Idiopathic myelofibrosis is usually associated with increase in mean vascular density. Increase in mean vascular density,megakaryocytic clustering and intrasinusoidal haematopoiesis are considered to be the pre-existing events leading to reticulin fibrosis and splenomegaly.

Hence studying the Mean vessel density by using markers such as CD34,CD31 and Factor VIII and correlating MVD with fibrosis, splenomegaly may be helpful in evaluating the disease progression and patients survival.

**ANNEXURE 1**

**PROFORMA**

**COIMBATORE MEDICAL COLLEGE**

**DEPARTMENT OF PATHOLOGY**

**COIMBATORE**

**Particulars of the patient:**

Name: Hospital:

Case no: Date:

Age/sex: I.P No:

Address: Ward no:

Occupation: Religion:

**Presenting complaints and duration:**

Weakness/Dyspnoea/Palpitation/Guiddiness/Angina+/-

Fever/Sweats/ Abdominal pain/Infection+/-

Purpura/Ecchymosis/Bleeding diathesis/Skin infections/Parasthesia+/-

**Past history:**

Previous history of anemia

Transfusions +/-,drugs +/-,liver diseases +/-,chronic diseases,

Exposure to radiation +/-,chemicals +/-.

**Family history:**

Anemia +/-,bleeding diathesis +/-,malignancy +/-,recurrent jaundice +/-,

**Personal history:**

Diet: appetite: bowel / bladder habits:

Sleep:alcohol intake : smoking

**Menstrual history:**

**General physical examination:**

Built:  nourishment: conscious: weight

Pulse :RR :BP:  febrile/afebrile:

Pallor: jaundice:cynaosis: clubbing:

Lymphadenophy: edema: mouth: skin:

**Systemic examination:**

P/A: hepatomegaly: splenomegaly: ascites:

CVS:RS:CNS: musculo-skeletal-bone pains +/-:

**Clinical diagnosis:**

**Investigations:**

1.Complete haemogram(autoanalyser)

| SI.no | Tests | Observed value |
|---|---|---|
| i. | Hb% | |
| ii. | RBC count | |
| iii. | WBC count | |
| iv. | Platelet count | |
| v. | PCV | |
| vi. | MCV | |
| vii. | MCH | |
| viii. | MCHC | |
| ix. | Retic count. | |

**2.Peripheral smear:**

RBC:

WBC:

Platelets:

Parasites:

Impression:

**3.Bone marrow study(Bone Marrow Aspiration no)**

Aspirate:

Cellularity:

Myeloid:eythroid rartio:

Erythropoiesis:

Leucopoiesis

Megakaryopoiesis:

**4.Bone marrow trephine(HPE NO)**

Cellularity:

Architecture:

Presence of fibrosis:

Focal lesions:

**5.Reticulin stain:**

Grading of fibrosis

**6.Spleen Size:**

**7.Mean Vascular Density:**

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**ANNEXURE 1**

**PROFORMA**

**COIMBATORE MEDICAL COLLEGE**

**DEPARTMENT OF PATHOLOGY**

**COIMBATORE**

**Particulars of the patient:**

Name: Hospital:

Case no: Date:

Age/sex: I.P No:

Address: Ward no:

Occupation: Religion:

**Presenting complaints and duration:**

Weakness/Dyspnoea/Palpitation/Guiddiness/Angina+/-

Fever/Sweats/ Abdominal pain/Infection+/-

Purpura/Ecchymosis/Bleeding diathesis/Skin infections/Parasthesia+/-

**Past history:**

Previous history of anemia

Transfusions +/-,drugs +/-,liver diseases +/-,chronic diseases,

Exposure to radiation +/-,chemicals +/-.

**Family history:**

Anemia +/-,bleeding diathesis +/-,malignancy +/-,recurrent jaundice +/-,

**Personal history:**

Diet: appetite: bowel / bladder habits:

Sleep:alcohol intake : smoking

**Menstrual history:**

**General physical examination:**

Built:  nourishment: conscious: weight

Pulse :RR :BP:  febrile/afebrile:

Pallor: jaundice:cynaosis: clubbing:

Lymphadenophy: edema: mouth: skin:

**Systemic examination:**

P/A: hepatomegaly: splenomegaly: ascites:

CVS:RS:CNS: musculo-skeletal-bone pains +/-:

**Clinical diagnosis:**

**Investigations:**

1.Complete haemogram(autoanalyser)

| SI.no | Tests | Observed value |
|---|---|---|
| i. | Hb% | |
| ii. | RBC count | |
| iii. | WBC count | |
| iv. | Platelet count | |
| v. | PCV | |
| vi. | MCV | |
| vii. | MCH | |
| viii. | MCHC | |
| ix. | Retic count. | |

**2.Peripheral smear:**

RBC:

WBC:

Platelets:

Parasites:

Impression:

**3.Bone marrow study(Bone Marrow Aspiration no)**

Aspirate:

Cellularity:

Myeloid:eythroid rartio:

Erythropoiesis:

Leucopoiesis

Megakaryopoiesis:

**4.Bone marrow trephine(HPE NO)**

Cellularity:

Architecture:

Presence of fibrosis:

Focal lesions:

**5.Reticulin stain:**

Grading of fibrosis

**6.Spleen Size:**

**7.Mean Vascular Density:**

# MASTER CHART

| S. No | HPE.No | Age / Sex | HPE Diagnosis | Reticulin Fibrosis (Grade) | Category | WBC Count* /cu.mm | Platelet Count*/ cu.mm | Hb (gm%) | Anisopoikilocytosis | Tear Drop Cells | Giant Platelet | Bone Marrow Cellularity | Abnormal megakaryocytes | Meg.cluster | Intrasinusoidal hematopoiesis | Presenting feature | Splenomegaly | Lymph Node | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 367/11 | 17/M | Primary Myelofibrosis | II | Primary | 2900 | 34000 | 5.1 | Present | Present | Present | Increased | Present | Present | Present | Abd.pain | Present | - | |
| 2 | 1972/11 | 72/M | Primary Myelofibrosis | II | Primary | 3800 | 59000 | 6.0 | Present | Present | Present | Increased | Present | Present | Present | pallor | Present | - | |
| 3 | 2369/11 | 56/M | Primary Myelofibrosis | III | Primary | 3500 | 87000 | 4.2 | Present | Present | - | Increased | Present | Present | - | pallor | Present | - | |
| 4 | 9503/11 | 52/F | Primary Myelofibrosis | II | Primary | 6900 | 95000 | 8.0 | Present | - | - | Increased | Present | - | - | pallor | Present | - | |
| 5 | 1395/11 | 65/M | Primary Myelofibrosis | III | Primary | 2400 | 67000 | 6.8 | Present | Present | - | Increased | - | - | - | pallor | Present | - | |
| 6 | 2654/11 | 58/F | Primary Myelofibrosis | II | Primary | 3700 | 69000 | 7.3 | Present | Present | - | Increased | Present | - | - | Fatiguability | Present | - | |
| 7 | 1475/11 | 51/M | Primary Myelofibrosis | III | Primary | 1800 | 47000 | 4.9 | Present | Present | - | Decreased | - | - | - | No symptom | Present | - | |
| 8 | 4914/11 | 33/F | SLE | II | Secondary | 8650 | 190000 | 9.0 | - | - | - | Normal | - | - | - | - | - | - | |
| 9 | 2491/11 | 65/M | Lymphoma (NHL) | I | Secondary | 7300 | 220000 | 9.2 | - | - | - | Increased | - | - | - | - | - | Enlarged | |
| 10 | 1401/11 | 55/F | SCC TONSIL | III | Secondary | 3800 | 73000 | 6.3 | - | - | - | Decreased | - | - | - | - | - | - | |
| 11 | 2303/11 | 40/F | AML | I | Secondary | 83000 | 68500 | 8.2 | - | - | - | Increased | - | - | - | - | - | - | |
| 12 | 41/12 | 48/M | NHL | II | Secondary | 8400 | 370000 | 11.2 | - | - | - | Increased | - | - | - | - | - | - | |
| 13 | 875/11 | 75/F | AML | I | Secondary | 120000 | 76000 | 10.6 | - | - | - | Increased | - | - | - | - | - | - | |
| 14 | 5176/11 | 50/F | NHL | I | Secondary | 6300 | 120000 | 11.0 | - | - | - | Increased | - | - | - | - | - | Enlarged | |
| 15 | 4021/11 | 15/M | Tuberculous Granuloma | I | Secondary | 7900 | 340000 | 10.8 | - | - | - | Normal | - | - | - | - | - | - | |
| 16 | 255/11 | 77/F | CLL | II | Secondary | 63000 | 280000 | 11.0 | - | - | - | Increased | - | - | - | - | - | - | |
| 17 | 8695/11 | 70/M | Multiple Myeloma | I | Secondary | 8600 | 220000 | 9.4 | - | - | - | Increased | - | - | - | - | - | - | |
| 18 | 5526/11 | 42/M | Granulomatous Lesion | II | Secondary | 9200 | 170000 | 10.7 | - | - | - | Decreased | - | - | - | - | - | - | |
| 19 | 3514/12 | 44/F | Metastatic Carcinoma Deposit | I | Secondary | 8900 | 240000 | 6.0 | - | - | - | Normal | - | - | - | - | - | - | |
| 20 | 3098/12 | 42/F | Metastatic Carcinoma Deposit | I | Secondary | 6800 | 345000 | 8.4 | - | - | - | Normal | - | - | - | - | - | - | |
| 21 | 2495/11 | 60/M | Primary Myelofibrosis | I | Primary | 4400 | 74000 | 8.7 | - | - | - | Decreased | - | - | - | pallor | Present | - | |
| 22 | 1760/11 | 60/M | AML | II | Secondary | 96000 | 110000 | 9.8 | - | - | - | Increased | - | - | - | - | - | - | |
| 23 | 2110/11 | 33/F | CML | I | Secondary | 140000 | 80000 | 10.9 | - | - | - | Increased | - | - | - | - | Present | - | |
| 24 | 886/11 | 54/F | MDS | I | Secondary | 3600 | 83000 | 8.0 | Present | - | - | Increased | - | - | - | - | - | - | |
| 25 | 3302/11 | 46/M | CML | I | Secondary | 160000 | 65000 | 8.5 | - | - | - | Increased | - | - | - | - | Present | - | |

# KEY TO MASTER CHART

WBC – White blood cells.

Hb - Hemoglobin.

NHL – Non hodgkins lymphoma.

SLE – Systemic lupus erythematosis.

SCC – Squamous cell carcinoma.

AML – Acute myeloblastic leukemia.

CLL – Chronic lymphocytic leukemia.

MDS – Myelodysplastic syndrome.

# A CORRELATIVE STUDY ON BONE MARROW ANGIOGENESIS WITH BONE MARROW FIBROSIS AND SPLENOMEGALY


## ABSTRACT

Myelofibrosis is defined as the pathological process characterised by increased deposition of collagen type I and type III.Marrow fibrosis usually results from the stimulation of fibroblast by its growth factors.Fibroplasia is associated with increased blood flow through the marrow substance.Examination of well prepared smears often yields many important informations.The initial primitive hematopoiesis gives rise only to erythroid precursors and macrophages.Fibrosis is not unique to Primary idiopathic myelofibrosis.It occurs in wide variety of diseases including Acute leukemias,Myelomas,Lymphomas and Metastatic deposits especially from breast and prostate.Idiopathic myelofibrosis is characterised by increase in bone marrow reticulin fibrosis and proliferation of blood vessels(Neoangiogenesis – Mean vessel density).The study included total of 25 cases of which eight were cases of primary myelofibrosis.Most of the cases of secondary myelofibrosis were cases of leukemias and lymphomas.IMF was mostly diagnosed in fifth to sixth decade. About 75% of the cases presented with pancytopenia.Increase in marrow cellularity is associated with increase in MVD.Increase in fibrosis is associated with decrease in cellularity.Increase in the spleen size(Extramedullary hematopoiesis) is independent of all the factors.Vascular proliferation is considered to be a mother event in causing splenomegaly and disease progression.



## KEY WORDS

Myelofibrosis,Fibroplasia,Bonemarrow,Reticulinfibrosis,Meanvessel density,Splenomegaly.